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STUDIES ON THE SUBCOMMISSURAL ORGAN IN SOME DOMESTIC ANIMALS

WITH REFERENCE TO SECRETORY PHENOMENA

BY

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VOL. 36 1958 SUPPLEMENTUM 9

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HELSINKI 1958

Suomalaisen Kirjallisuuden Kirjapaino Oy Helsinki 1958

PREFACE

The present investigation has been carried out partly at the Department of Anatomy of the Veterinary College and partly at the Department of Anatomy of the University of Helsinki. Favourable conditions for the commencement of this work were created by the Chief of the Department of Anatomy of the Veterinary College at that time, Professor *V. M. Klemola*, Ph.D., D.Agr. & For., Vet., who placed all the facilities of his institute at my disposal. Up to his unexpected death, he followed the progress of my work with unwearying interest and aided it by ample advice and directions.

In this connection I have particularly to mention Assist. Professor *E. Kivalo*, M.D., in the Department of Anatomy of the University of Turku, who suggested the subject of this study and to whom I am indebted for the advice and guidance he has given me, without ever sparing his time, during the planning and performance of the work and during the preparation of the manuscript. To him I offer my deeply felt thanks.

I wish to thank Professor *O. Eränkö*, M.D.,Ph.D., who has kindly allowed me to pursue this work also in the Department of Anatomy of the University of Helsinki. He has given much of his time for talks concerning the planning of the work, he has guided it with valuable advice and has perused the manuscript, for which my most sincere thanks are due to him.

To Docent *A. Telkkä*, M.D., I am obliged for his kindness in making available to me some difficultly obtainable reagents.

To the Deputy Director, Mr. *B-O. Engdahl*, Vet., the Director-in-Chief, Mr. *E. Huhtala*, Vet., Assist. Professor *S. Paatsama*, D.V.M., Professor *V. Rislakki*, D.V.M., Professor *P. I. Stenius*,

D.V.M., and Professor *O. Turpeinen*, M.D., Ph.D., I wish to express my thanks for the interest they have shown towards my work and the aid they have given me during its progress.

I also wish to state my gratitude to Miss *Anitra Sandberg*, who has performed a great part of the technical work associated with the investigations, to the Laboratory Master, Mr. *T. Pitkänen*, who has assisted me in the procuring of the material and its preparation, and to the Preparator, Mr. *E. Muona*, who has carried out most of photographic work.

My grateful recognition is also due to Mr. *U. Attila*, M.Sc., who has translated the manuscript into English. My thanks are also due to Assist. Professor *T. F. Mustanoja*, D.Ph., for the revision of the English text.

Finally I owe a great debt of gratitude to my sister for her constant patience and help in the course of the study.

Economically, the work has been subsidized by a grant received from the Science Committee of the State.

Helsinki, September 1958.

Seppo Talanti

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INTRODUCTION

The development that has occurred in histological and histochemical technics has delivered new, efficient tools into the hands of investigators for an even more detailed study of the structural peculiarities of the cells and tissues. It has its significance also in regard to the investigation of the function of various organs since quite often the structure of an organ reflects its functional activity. This kind of an indirect investigation of the functional character is particularly important in the case of such organs where for purely technical reasons the elucidation of their function by the conventional physiological expedients is rendered difficult or altogether impossible.

Despite a great amount of work dedicated to the investigation of the subcommissural organ, our knowledge of the structure, the function and the physiological significance of this organ is still incomplete. It constitutes a specialized area of the ependyma located beneath the posterior commissure of the midbrain in the roof of the third ventricle, in the region where the ventricle suddenly narrows to become the cerebral aqueduct. One may say that this organ still constitutes one of the numerous problematic regions of the living organism. However, indications have been gained, particularly from investigations performed during the most recent decade, to the effect that secretory activity would occur in this organ.

The conception of secretory activity taking place in the central nervous system is not new in itself. One has been aware for a long time already that the cerebrospinal fluid is the product of secretory activity in the chorioid plexus (e.g. Pettit and Girard 1902) More recently, the idea of the neurosection proper, occurring in

the ganglion cells of the central nervous system, has been formed (Speidel 1919, Scharrer 1928). By employing certain staining methods hand in hand with physiological investigations convincing proof has been obtained of the secretory activity present in certain nuclei of the hypothalamus (e.g. Bargmann 1957), although this problem is still subject to lively discussion. It is just on the basis of the results from investigations carried out with the aid of such methods that also the opinion of the glandular character of the subcommissural organ is gaining strength.

The purpose of the present work was to study with the aid of histological and histochemical methods the subcommissural organ of some of the commonest domestic animals; the ox, the horse, the pig, and the dog. The following objectives of investigation were set for this work:

1) To provide a description of the histology and certain histochemical properties of the subcommissural organ of the animals examined.

2) To look for such structural characteristics in the subcommissural organ of the animals examined which may be significant on judging the function of the organ and particularly its potential glandular character,

3) To localize in the subcommissural organ of the animals examined its potential secretory activity in order to furnish an answer to the question of a) where such secretion occurs, and b) whither the secretion is directed,

4) To determine, with the aid of histochemical methods, the character of the secretion that may be encountered in the subcommissural organ of the animals examined and,

5) To elucidate the relation of the subcommissural organ of the animals examined to the so-called Reissner's fibre.

REVIEW OF THE LITERATURE DEALING WITH THE SUBCOMMISSURAL ORGAN AND ITS RELATION TO REISSNER'S FIBRE

Dendy (1902) was the first to consider the subcommissural organ (henceforth to be referred to as S.C.O.) as an independent organ with structural properties and even tasks differing from those of the rest of the cerebral ventricle walls. He even gave it a name »ciliated grooves». Dendy's observations are based on investigations performed with *Ammocoetes*. With this animal he observed on the lower surface of the posterior commissure small depressions covered over by ciliated, cylindrical cells, which he thought to have the task of taking care of the fluid circulation in the cerebral ventricles.

Even prior to this investigation of Dendy the literature contains some mention of the S.C.O. As a rule they are based on observations made in connection with investigations on the ependymal tissue lining the cerebral ventricles. Stieda (1870) in his investigations with mice, Fulliquet (1886) with *Protopterus*, Rabl-Rückart (1887) with reptiles, frogs, and birds, and Gaupp (1897) with frogs observed that on the lower surface of the posterior commissure the ependymal cells become tall and cylindrical, i.e., typical ependymal cells of the S.C.O. according to their descriptions.

Also in some investigations concerning the so-called Reissner's fibre the S.C.O. can be found mentioned about the turn of the century. Reissner (1860) was the first to describe this fibre with *Petromyzon fluviatilis* as a thin formation resembling the axis cylinder and running in the central canal of the spinal cord; later on varying opinions were expressed about this formation. For instance, Stieda (1868) considered the fibre an artifact produced on coagulation of the contents in the central canal of the spinal cord through the effect of the fixing fluids. Studnička

(1899) on the other hand, who employed mainly *Petromyzon* for his investigations, regarded it as an actual formation and thought that it originated as a secretion of the ependyma in the central canal. Sargent was the first to suggest an organic and functional connection between Reissner's fibre and the S.C.O. His extensive investigations (Sargent 1900, 1901, 1904) used fishes, birds, reptiles, and also mammals as their material. He presented the ultimate conclusion that the function of the S.C.O. was to support Reissner's fibre, which latter formation transmits optical reflexes and thus constitutes a nerve fibre. Sargent gave the name of *recessus mesocoelicus* to the indentation extending dorsally, which is encountered with nearly all animals in the region of the S.C.O. This name has been retained in later investigations as well.

Kappers (1907) observed that the lower surface of the posterior commissure of *Amia calva* and *Lepidosteus osseus* was covered by typical S.C.O. cell tissue. Marburg (1908) was the first to make observations relating to the S.C.O. of man in connection with an investigation on the pineal body. Typical cell tissue was found in man, too, on the lower surface of the posterior commissure.

In addition to his above-mentioned investigation, Dendy published further studies concerning the S.C.O. in 1909 and 1910. He opposed Sargent's opinion of the task of the organ, disputing the nerve character of Reissner's fibre. He also refuted his earlier view of the functional character of the organ. Now, he considered that the organ together with Reissner's fibre constitutes a mechanism controlling the motions of the body, particularly the flexions.

Nicholls, in 1909, concurred with Dendy's opinion of the function of the S.C.O., and in 1910 Dendy and Nicholls together published an investigation in which the name »subcommissural organ» was suggested for the organ; this name has then been retained and is used even at the time being. They described the organ as being composed of two strips made up of cylindrical epithelium and joining to form a more or less pronounced fold. In the lower animals the organ is well-developed but in man it is only rudimentary, excepting the foetus, in which it shows a fairly high degree of development. The said investigators also

described the structure of the S.C.O. of the mouse, cat and chimpanzee. The *recessus mesocoelicus* was present in all the animal species examined. Later, Nicholls (1912, 1913, 1917) published further investigations in which he endeavoured to prove the correctness of his opinion of the task of the S.C.O.

The first, as far as is known, to assume that glandular secretion takes place in the S.C.O. was Bauer-Jokl (1917). She studied a great number of different animals, among others the ox, pig, and dog. Man was also included in her material of investigation. She found that the various animals display great differences in degree of development and that the organ is rudimentary in man. The *recessus mesocoelicus* was present in all the animals examined. Bauer-Jokl based her opinion of the glandular character of the S.C.O. on the peculiarities found by her in the structure of its ependymal cells. The cells were cylindrical, resembling goblet cells, and amorphous masses could be demonstrated on their free surface, entangled with the cilia of the cells. The investigator interpreted these masses as consisting of a secretion originating from the cylindrical cells. She pointed out the close connection between the S.C.O. and the pineal body, stressing the fact that both organs regress in man during extrauterine life. Bauer-Jokl also called attention to the subependymal tissue of the S.C.O., which she described as a glia cell tissue rich in cells.

Marburg (1920) observed on the basis of the investigation concerning the parietal organ that the pineal body and the S.C.O. are two completely distinct organs although a certain kind of reciprocity was observable in their structure with some animals. A large pineal body was associated with a small S.C.O., and *vice versa*. However, this was not always observable, for instance not in the ox and the horse. The organ obtains its innervation from the *fasciculus subcommissuralis*. Marburg judged the task of the organ to be that of controlling the secretion of cerebrospinal liquor. The receptor part in this controlling mechanism would be Reissner's fibre, the changes in its tension resultant upon changes of the liquor pressure causing changes in the organ itself.

A rather remarkable contribution to the investigation of the S.C.O. has been made by Kolmer (1919, 1921, 1925, 1930, 1931). He has studied a very extensive animal material including, among others, all domestic animals, and he has given an accurate and

detailed description of the S.C.O. of the various species. Kolmer, too, found that the degree of development varies greatly with the different species and he also verifies the postnatal regression of the S.C.O. in man. All animals displayed tall, columnar cells in the surface tissue of the organ. The cell nucleus had a basal location and the cells bore a cilium with diplosomes. In some cells »drop-like elements (tropfenartige Elemente)» were noted on the surface of the cell. Although no organic connection is demonstrable, according to Kolmer, between Reissner's fibre and the S.C.O., at least not distinctly, he is of the opinion that they form together functionally the so-called »sagittal apparatus», a kind of sensory mechanism participating in the reflexes affecting the movements of the spine and the body in general. The activity was likened to that of the organ of Corti and the tectorial membrane. However, Kolmer did not observe any primary nor secondary sensory cells in the S.C.O., but he found nerve fibres traversing the organ and considered this to be in favour of the described functional character. Kolmer assumed a very strict opinion against the glandular character of the organ. The drop-like masses resembling of a secretion found by himself he interpreted as representing the same substance which was also encountered around the hypophysis, for which reason they could not have been secreted by the ependyma according to Kolmer. The cells enlarged to a goblet-like shape, which he found in the ependyma and which would speak in favour of a secretory function, Kolmer explained as indicating unsatisfactory fixation.

At about the same time as Kolmer's investigations, an investigation on the ependymal cell tissue based on an extensive animal material was published by Agduhr (1922). In this work he also described the structure of the S.C.O. and observed that Reissner's fibre is connected with it by thin threads in such a degree indeed that in the S.C.O. of the ox the branches of such threads could be seen to penetrate into the interstices of the ependymal cells. Agduhr considered the task of Reissner's fibre to be obscure, although he concurred with the opinion pronounced by Jordan (1919) that the fibre acts not in the manner of a nerve. On the function of the S.C.O. Agduhr (1932) says: »A fuller knowledge of the function of this organ is lacking but different possibilities have been discussed, e.g. that it may be a glandular organ, an

organ of sense especially for regulating movements in the ventricular fluid».

Krabbe (1916, 1925, 1933) in his comprehensive investigations, has described the structure of the S.C.O. on the basis of a very extensive animal material, including e.g. all domestic animals. Particularly in cattle and dogs he found this organ to be well-developed. He verified numerous observations of Kolmer's and of other earlier investigators, concerning the structural details of the organ. However, he did not observe the amorphous »secretion» mentioned by Bauer-Jokl, and he found the protoplasm of the ependymal cells to be homogeneous for the most part although varying staining intensity could be noted in different cells. Krabbe interpreted this as resulting from different functional phases. He did not find any nerve fibres in the area of the organ. Krabbe also gave a detailed description of the subependymal tissue, designated by him as the hypendyma. In this tissue he observed cells containing little protoplasm and resembling glia cells. The tissue was rich in nuclei and contained neuroglia elements in abundance. It was profusely vascularized and in its deeper layers some nerve fibres originating in the posterior commissure could be observed. With regard to the function of the organ, Krabbe only presented some possibilities without taking any definite view in this question. According to him, the organ could be the motor producing a flow of the cerebrospinal fluid, or it could absorb something from the fluid. Secretion was mentioned as a possibility, though not a very likely one.

Jordan (1919, 1925), on investigation of the S.C.O. and Reissner's fibre in lower animals, observed that the fibre is attached to the surface of the organ. He thought Reissner's fibre to consist of elastin because it was stained by safranine. Jordan did not concur with the idea that fibre and organ would constitute an intracerebral sensory organ. In an experimental study he found that severing Reissner's fibre did not produce any observable changes in the behaviour of the test animals.

In the ependymal cells of the human S.C.O., Puusepp and Voss (1924) found changes which they interpreted as resulting from secretion phases. On the surface of the organ masses were observed, which probably consisted of material secreted by the organ. According to the authors the organ regresses in man so

that obviously no more activity is observable after 30 years of age. The secretion was assumed in some way to affect the brain, possibly the growth of the brain.

Berblinger (1926) and Ishikawa (1927) assumed that a certain kind of relationship exists between the pineal body and the S.C.O. Thus, they assigned a secretory function to the S.C.O. Ishikawa substantiated his opinion by his observation with the dog that differences occur in the development of the organs between the different sexes, a large pineal body going together with a small S.C.O., and *vice versa*.

Pastori (1927), too, related the S.C.O. closely with the pineal body. He, for instance, found similar cells in both organs with cattle and horses. According to him, small pineal body cell islets could be observed in the S.C.O. of horses; in cattle, moreover, pineal body tissue occurred in the dorsal part of the organ.

Saitta (1930) maintained that the S.C.O. would not be active at all in adult animals. According to him, it is some kind of a rudimentary residue of a larger organ during the earlier phylogenetic phases of development.

Turkewitsch (1933, 1936 a, 1936 b, 1937 a, 1937 b) has presented a series of studies relating to the development of the S.C.O. In his investigations with man, ox, sheep and rabbit he arrived at the result that the pineal body and the S.C.O. stand very close to each other embryologically. He considered the subependymal tissue, the hypendyma, a highly central part of the S.C.O., and in the animals examined, among others in cattle, he thought the hypendyma to be a formation homologous with the posterior part of the pineal body in man.

Pesonen (1940 a, 1940 b) studied the S.C.O. in the guinea pig and in man. In the columnar cells of the ependyma in the guinea pig he observed a drop-like, partly granular mass which was also encountered on the free surface of the organ. Some cells were enlarged to goblet shape. Pesonen interpreted these findings as well as those of the profuse vasculature of the organ and its general structure resembling of a tubular gland as being indicative of glandular activity. The secretion was directly discharged into the cerebral ventricle. There were no sensory cells in the area of the organ. Reissner's fibre arose immediately from the surface of the ciliated ependymal cells of the organ. In man, the organ

was well-developed in the foetal period but immediately upon birth the ependyma was clearly regressing. In the adult no more than traces of the S.C.O. remained, and Reissner's fibre was lacking.

Pesonen and Setälä (1941) studied the adjoining cell tissue above the S.C.O. in man. The tissue was found to be profusely vascularized and rich in cells. It was made up of three types of cells: cells with a large, round nucleus, with a small, round nucleus, and with an oval nucleus, respectively. This tissue was thought to have some function which it performed already during intrauterine life but no definite opinion was stated as to the character of this function.

Reichold (1942) investigated the S.C.O. of some mammals and sauropsids. In the cells of the ependyma he observed fluid vacuoles, and all cells were ciliated. On the free surface distinct »granular-mucous masses (körnig-schleimige Massen)» were present and the author interpreted them as secretion. According to him, the hypendyma was made up of cell elements of a macroglia and microglia type, forming a narrow, reticular tissue layer between ependyma and posterior commissure.

Up to this point the S.C.O. had been investigated with a very remarkable number of species of animals. Investigations have also been made by Chiarugi (1932) with marsupials, Fuse (1936) with aquatic mammals, Suzuki (1938) with several primate species and Yamada (1938) with *Echidna*. Bargmann (1943) mentioned in his comprehensive survey that the S.C.O. and Reissner's fibre are absent only in adult man, *Sorex*, *Crossopus* and in the hedgehog.

The structure of Reissner's fibre and its attachment to the S.C.O. has been studied by Eberl-Rothe (1951). By means of his special technique he attempted to isolate the fibre in some vertebrates. He observed that the fibre is attached at its cranial end to the S.C.O. and that the initial part of the fibre is hollow, tube-like. In the fibre itself the investigator was able to find cellular elements.

An epoch in the investigation relating to the S.C.O. was marked by the development of the new, selective histological staining methods indicating the hypothalamo-hypophysial neurosecretory phenomena. Quite soon material stained by these techniques was observed also in the cells of the S.C.O. All the inves-

tigators who have studied the S.C.O. by these methods concur with the opinion that secretion is at least one of the functions of this organ.

Bargmann and Schiebler (1952) observed that the S.C.O. of the dog and cat contained material reacting positively to the chrome-alum-haematoxylin-phloxine stain (Gomori 1941 a). Such material was observed in the region of both ependyma and hypendyma. Reissner's fibre reacted positively but no organic connection between the fibre and the organ could be demonstrated. The material also reacted positively to the periodic acid-Schiff technique. From this the inference was drawn that the stained material, i.e., the secretion of the S.C.O. might possibly be glycoprotein.

Simultaneously with the investigation just mentioned, Wislocki *et al.* commenced their extensive series of investigations dealing also with the structure and histochemical properties of the S.C.O. and Reissner's fibre in small rodents used as laboratory test animals (Wislocki and Leduc 1952 a, 1952 b, Leduc and Wislocki 1952, Wislocki and Leduc 1954, Wislocki, Leduc and Mitchell 1956). The investigations revealed that the S.C.O. of the animal species examined contained material stained by the chrome-alum-haematoxylin-phloxine method and by the aldehyde-fuchsin method. The material was periodic acid-Schiff-positive and of lipoidal character. In the organ strong enzymatic activity of certain enzymes was also observed. Reissner's fibre was similar in the small rodents and in the rhesus monkey in regard to its histochemical properties to the material in the organ reacting positively to the staining, and it was thought to consist of secretion of the organ. However, the possibility was not refuted that the secretion might even contain something aside from the matrix of Reissner's fibre. Wislocki and Roth (1958) observed that although Reissner's fibre is lacking in the human foetus, the S.C.O. of the foetus contained »secretion» acquiring a stain by the chrome-alum-haematoxylin-phloxine and aldehyde-fuchsin methods, which was located in the same region as the periodic acid-Schiff-positive material.

In 1952 also Mazzi observed, in the S.C.O. of lower vertebrates, material reacting positively to the chrome-alum-haematoxylin-

phloxine staining. The material was obviously periodic acid-Schiff-positive and probably consisted of glycoprotein. Reissner's fibre was found to have the same staining properties and the investigator concurred with the opinion of Wislocki *et al.* that the fibre was composed of the secretion of the organ. Mazzi stated nothing on the potential tasks of the fibre. With *Rana agilis*, Mazzi (1954) was able to show that the stainable secretory material in the S.C.O. appears earlier than the material in the hypothalamo-hypophysial neurosecretory system stainable by the corresponding methods.

Stutinsky (1953) observed in *Anguilla vulgaris* that the S.C.O. contained material staining with the chrome-alum-haematoxylin-phloxine method. Similarly stainable was also Reissner's fibre, which attached to the anterior part of the S.C.O.

Oksche (1954), on investigating an *Anura* species, observed that it possessed material stainable by the chrome-alum-haematoxylin-phloxine method which was located either apically or basally in the ependymal cells of the S.C.O. From this the inference could be drawn that the organ acts as a gland secreting either into the cerebral ventricle or into the capillary network, which is in immediate contact with the basal parts of the surface cells. Later on, Oksche (1956) published his investigation comprising animals from *Cyclostoma* to man. He forwarded the assumption that in the higher animals the secretion is mainly directed into the cerebral ventricles, while the discharge into the capillary network has small significance. He considered the secretion to be a mucopolysaccharide or a mucoprotein.

Arvy, Fontaine and Gabe (1955) found the material stainable with the chrome-alum-haematoxylin-phloxine method in the S.C.O. to be independent on simultaneously occurring changes in the quantity of the similarly stainable material in the hypothalamo-hypophysial system. These observations were made with *Salmo salar*.

Okada (1955) observed in *Bufo vulgaris* »secretory material» in its S.C.O., which travelled by a special »secretory path» into the subarachnoidal space through the posterior commissure.

Grignon (1956) observed in the S.C.O. of the chicken embryo material reacting positively to chrome-haematoxylin and to

aldehyde-fuchsin. On the basis of the distribution of this material he forwarded the hypothesis that the ependymal cells in the S.C.O. of the chicken embryo are capable of bipolar secretion.

Murakami, Ban and Aiura (1957) observed in *Gecko japonicus* material reacting to aldehyde-fuchsin and to the periodic acid-Schiff method in the S.C.O. The investigators think that this material considered by them to be a secretion is discharged either directly into the circulation or in the manner presented by Okada through the posterior commissure into the subarachnoidal space. Reissner's fibre was not observed with the *Gecko*.

Fleischhauer (1957), on investigating the S.C.O. of the tortoise, observed in the organ material stainable with chrome-haematoxylin, which could also be fixed with alcohol. He also noted a distinct connection with Reissner's fibre. Moreover, he observed periodic acid-Schiff-positive material and fat in the apical parts of the ependymal cells.

The assumption that the materials stainable in a similar manner in the hypothalamo-hypophysial system and in the S.C.O. would be similar in their physiological character has instigated some experimental investigations. Wingstrand (1953) observed that the S.C.O. of the chicken embryo contains chrome-haematoxylin-positive material as early as in a six-day old embryo. He prepared extracts from it and tested their effect upon the water balance of the organism of the toad, *Bufo bufo*, although without any positive results. He arrived at the conclusion that Reissner's fibre is probably formed of the secretion of the S.C.O.

Gilbert (1956, 1957) performed an experimental investigation with the rat by electrocoagulative destruction of its S.C.O., injecting another series of the animals with homogenates prepared from the organ. The animals deprived of their S.C.O. died while the animals treated with injections showed reduced water consumption in varying degrees. The active principle was very sensitive and did not tolerate alcohol or acetone treatment. On the other hand active homogenates were obtained with the aid of water or isotonic saline. Gilbert arrived at the conclusion that the S.C.O. is a secretory gland regulating thirst.

The S.C.O. has also been investigated by electron microscopy (Afzelius and Olsson 1957). The object of investigation was *Myxine*. The result of this study was that a secretory gland must be

concerned, discharging directly into the cerebral ventricle. The cells secreted apocrinally. All cells were ciliated. Reissner's fibre consisted of a homogeneous mass and no cavity could be observed in its initial portion. The cilia were thought to constitute some kind of mechanism driving the secretory product backward.

OWN INVESTIGATIONS

1. MATERIAL

The material consisted of S.C.O.'s of ox, horse, pig and dog, taken from individuals of both sexes of each animal species.

Cattle from which material was obtained were either of the Ayrshire or of the Finnish breed. Their age varied between two weeks and twelve years. Their majority consisted of animals to be slaughtered, and in these cases the material was not received for further treatment until 20 to 30 minutes after the animal's death. For the histochemical investigations also three calves were used, the material being then excised in chloral hydrate narcosis. The lapse of time from disruption of circulation to the further treatment of the material could be brought down to about three minutes in these instances.

All horses were animals to be slaughtered. They were of the Finnish breed and their ages varied between 3 and 15 years. The time between death and reception of the material for further treatment was about 30 to 40 minutes.

The pigs were either of the Yorkshire or of the home-bred Finnish breed. All pigs were less than one year of age. About 30 minutes passed from the animal's death to commencement of the further treatment of the material.

The material relating to the dog was obtained from dogs exterminated while healthy, or from healthy experimental dogs. The age of the animals varied between three months and 12 years. They were killed by magnesium chloride solution injected into the vein. The material was obtained from these dogs about ten minutes after the animal's death. The experimental dogs were anaesthetized with »Evipan-natrium» (Bayer) and the material was excised in deep narcosis, the lapse of time between disruption

of circulation and fixing or freezing of the material amounting to about 1 to 3 minutes. Altogether four experimental dogs were used.

Altogether, the material employed in the investigations comprises the following numbers of S.C.O.'s: 42 of the ox, 12 of the horse, 36 of the pig, and 18 of the dog.

2. THE MICROSCOPIC STRUCTURE OF THE SUBCOMMISSURAL ORGAN

It is evident from the survey of literature presented in the foregoing that investigation relating to the structure of the S.C.O. has been carried out to remarkable extent even before the selective methods specially adapted to describe the neurosecretion were adopted. A great number of different animal species have been subjected to such investigations. The species concerned in the present work, too, have been studied before (e.g. Bauer-Jokl 1917, Kolmer 1921, Agduhr 1922, Krabbe 1925, Turkewitsch 1936 a, 1936 b). Results somewhat at variance with each other have been obtained in these investigations, as can also be inferred from the conclusions drawn from them with regard to the functional character of the organ. In the present work the attempt has been made, employing mainly the same procedures as in the investigations cited above, to study such structural properties which have significance in regard to an assessment of the function of the S.C.O. No particular attention was paid to the differences between different species which might have been observed in regard to size, shape and topographic relations of the S.C.O. The present observations were rather concentrated on the microscopic structure of the organ, i.e., on the structure of its cells and their mutual relations in the S.C.O.'s of the animals examined.

Methods

The material was fixed in 10 % formalin, in 10 % neutral formalin or in Zenker's formol and embedded in paraffin in the usual manner. Sections were made at 3 to 10 micra in the sagittal, horizontal and frontal planes.

The following staining methods were employed: haematoxylin-eosin staining, Heidenhain's iron-alum-haematoxylin method, Hansen's iron-trioxide-haematein-eosin method, Weigert's resorcin-fuchsin method, van Gieson's haematoxylin-picrofuchsin method, and Davenport's silver impregnation method. All methods were employed in the manner presented by Romeis (1948).

Results

In all species of animals examined in this work two parts could be distinguished in the S.C.O., namely, the surface tissue, which will be referred to as the ependyma in the following, and the subependymal tissue or the hypendyma.

Ox

Ependyma. — The ependymal cells were found to be tall, columnar cells, forming a surface layer in the manner of a columnar epithelium. The height of the cells in the various parts of the organ was not greatly variable. In the lateral parts of the organ, the change of the ependyma of the S.C.O. into »common» ependyma was rather abrupt, the border-line forming a ridge-like elevation. In the cranial and caudal parts of the organ, on the other hand, the ependyma changed gradually without any sharp demarcation. The ependyma lining the cerebral ventricles elsewhere consisted of comparatively low, cubical cells in the ox as well as in the other animals examined.

A feature characteristic of the ependyma of the ox's S.C.O. was its strong folds observable in the entire region of the organ, which gave the ependyma an undulating appearance in the sections. The ependyma also formed numerous crypts of an extension and depth varying in the different parts of the organ. Most abundantly such crypt formation was encountered in the region of the *recessus mesocoelicus*, where the crypts produced an almost spongy structure of the tissue. In cross sections the crypts had the appearance of follicles covered by an ependyma of somewhat lesser height than that in the superficial parts of the organ (Fig. 1).

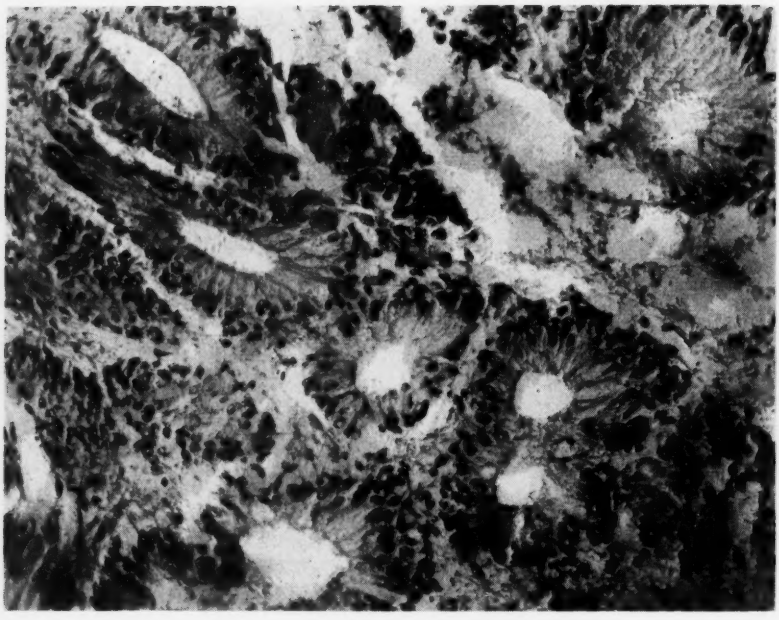

Fig. 1. Ox. — Section from the region of the *recessus mesocoelicus*. — Haematoxylin-eosin. Magnification ca. 180 ×.

The nuclei with a clearly distinguishable nucleolus in the ependymal cells were comparatively large and mostly tending towards an oval shape. They were located basally in the cells and formed a dense layer with multiple rows in the basal part of the ependyma.

On the free surface of the ependymal cells a distinct cilium was observable, with a diplosome at its base. The cilia were entwined to dense pleats intermingled with the mass encountered on the free surface. The apical part of the ependymal cells frequently penetrated as a club-shaped projection into the cerebral ventricle, as a result of which the surface of the organ had an uneven appearance. In some cells the apical part was distended to goblet shape. In the cytoplasm of nearly all cells a granular mass could be demonstrated, its quantity in the cells increasing towards the free surface (Fig. 2). This granular mass was occasionally also seen in the narrow basal, root-like cytoplasmic processes of the cells invading the subependymal tissue and forming,

Fig. 2. Ox. — Point of opening in the ependyma of a hypendymal duct. Below the depression formed by this opening, a cross section of a similar duct, indicated by arrows. Cytoplasmic, granular material in the ependymal cells; some cells are distended to goblet shape. — van Gieson's stain. Magnification ca. 660 ×.

in places, dense plaits observable particularly around blood vessels.

The surface of the organ carried similar granular mass occurring in droplets. This mass also filled the above-mentioned crypts formed by the ependyma. Reissner's fibre did not appear to have any direct organic connection with the surface of the S.C.O. It seemed to originate in the loose, granular mass on the surface of the organ. At some points Reissner's fibre was connected with the surface of the organ by a narrow zone of granular mass.

No nerve fibres could be demonstrated in the region of the

Fig. 3. Ox. — Hypendymal tissue with two cross sections of ducts, indicated by arrows. — van Gieson's stain. Magnification ca. 500 ×.

ependyma. On the other hand capillaries emerged in some places from the hypendymal tissue, running in the interstices of the ependymal cells.

Hypendyma. — The tissue layer was found to be comparatively abundant. In the region containing a great number of ependymal crypts the layer was very thin in some places.

The main part of the hypendyma presented a reticular, loose tissue with cells containing as a rule only scantily cytoplasm and possessing ramified processes by which the cells were in contact with each other. Two main types of cells could be distinguished. One type of cell had a small, dense, fairly round or somewhat oval nucleus and a scanty amount of cytoplasm, whereas in the other type the nucleus was oval, larger than in the preceding type and poorer in chromatin, and more cytoplasm was encountered in these cells.

In addition to the tissue constituents just described, the

hypendyma presented, in places even in great abundance, cell groups which the sections made in different directions proved as probably constituting the walls of tubular ducts (Fig. 3). Obviously these ducts had a very tortuous course so that it was possible to see both longitudinal and cross sections in one and the same section. The diameter of the tubes seemed to be roughly the same in the different regions of one and the same organ by ocular assessment. A small lumen could be distinguished in their middle, from which narrow capillary spaces penetrated in between the parietal cells, toward the periphery of the tube. The cells lining the walls of the tubes had conical shape in cross sections. They were in contact with each other at the periphery of the tube only, the above-mentioned capillary spaces separating them centrally. In the basal part of the cells no basal membrane could be observed. The nucleus of the cells was round or slightly oval, with a clearly distinguishable nucleolus. The cytoplasm of the parietal cells, particularly that in the parts closest to the lumen of the ducts, contained granular mass, its quantity being greatest immediately adjacent to the apical limit of the cell. The granular structure was highly distinct and the granules acquired a red stain particularly with haematoxylin-eosin and with van Gieson's method. The capillary spaces between the cells and the lumina of the ducts were filled with similar granular material.

The structures just described as ducts were mainly located in the region of the hypendyma, although ducts could also be observed here and there in the cell tissue of the ependyma (Fig. 2). Some ducts could be observed to open onto the surface of the ependyma or into the crypts formed by the ependyma. In some places the ducts penetrated into the interstices of the fascicles of the posterior commissure and some of them even traversed it.

In the hypendymal tissue a very great abundance of blood vessels could be noted, which formed a coherent network around the basal part of the ependymal tissue. Connective tissue fibres could be observed around the blood vessels and also here and there elsewhere in the region of the hypendyma.

In the hypendymal tissue delicate nerve fibres occurred, which seemed to have their origin in the nerve bundles of the posterior commissure (Fig. 4).

Fig. 4. Ox. — Nerve fibres in the hypendymal tissue. — Davenport's method. Magnification ca. 600 ×.

Horse

Ependyma. — The ependymal cells were found to be tall and columnar like those of the ox (Figs. 5 and 6). On the edges of the organ the tissue passed over without any sharp demarcation into the ependyma lining the cerebral ventricles. Even outside the boundary of the typical S.C.O. tissue proper, small cell islets could be observed here and there with similar cell tissue consisting of tall, columnar cells.

As a rule, the ependyma occurred as a uniform layer with only insignificant folds here and there in the region of the organ. Crypts could not be observed at all, at least none similar to those

Fig. 5. Horse. — Ependymal cells with basal cytoplasmic processes penetrating into the hypendyma and winding around blood vessels in places. — Haematoxylin-eosin. Magnification ca. 500 ×.

of the ox. The *recessus mesocoelicus* was well-developed but owing to the lack of crypts the tissue was not spongy and the ependyma merely formed a simple depression in the dorsal direction.

The ependymal cells were largely similar to those of the ox also in regard to their internal structure. Some cells distended to goblet shape could be noted and the cytoplasm of nearly all cells contained granular mass. The apical parts of the cells extended into the cerebral ventricle as club-shaped projections. A difference in comparison with the ox consisted of the basal root-like cytoplasmic processes which were singularly well-developed in the

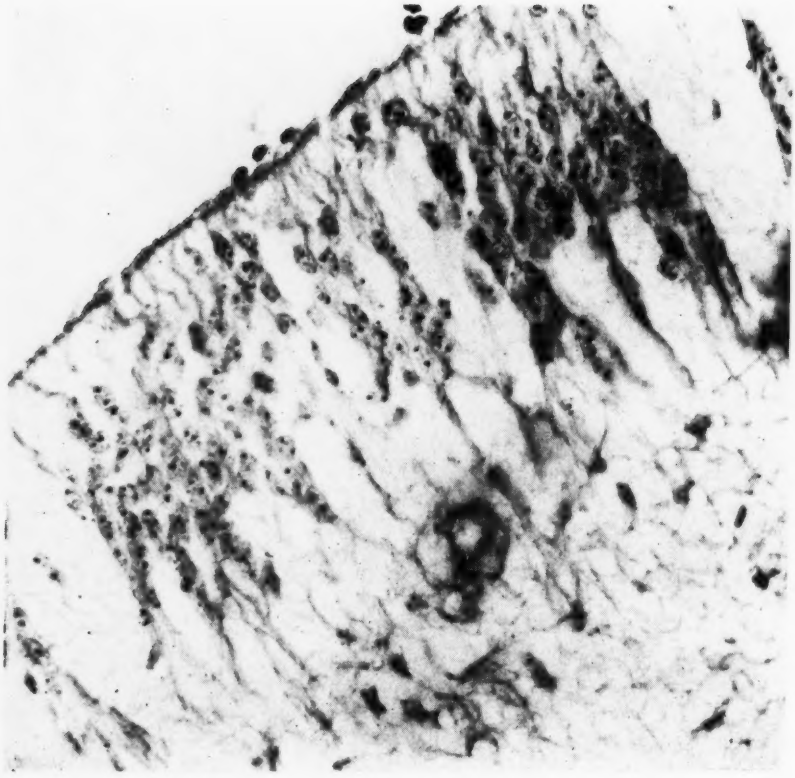

Fig. 6. Horse. — Ependymal cells with long basal processes. — Heidenhain's iron-haematoxylin. Magnification ca. 500 ×.

horse and penetrated deep into the hypendymal tissue (Figs. 5 and 6). The processes were ramified and formed plaits particularly around the hypendymal blood vessels. The cells bore a distinct cilium, the plaits formed by the cilia intermingling with the mass occurring in droplets on the surface of the organ and showing distinct granular structure in places.

Reissner's fibre seemed to originate in the above-mentioned loose mass on the surface of the organ. No immediate connection between the surface of the organ and the fibre could be noted.

In the ependyma capillaries could be observed occasionally between the ependymal cells. No nerve fibres could be demonstrated in this layer.

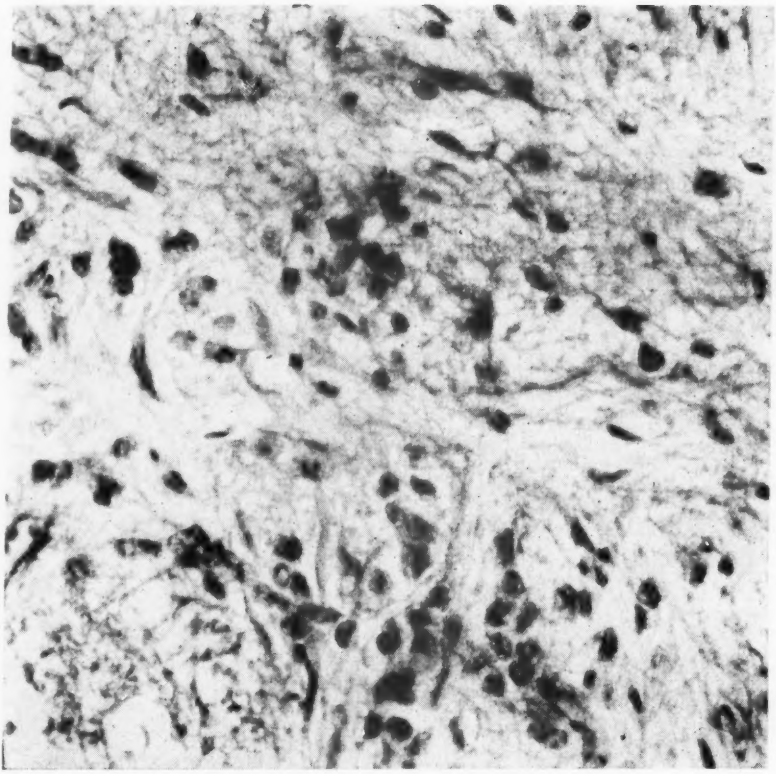

Fig. 7. Horse. — Hypendyma, in which two types of cells can be distinguished: cell with a large nucleus poor in chromatin, and cell with small, darkly staining nucleus. — Haematoxylin-eosin. Magnification ca. 600 ×.

Hypendyma. — The hypendyma was particularly well-developed. It was fairly uniform in thickness in the region of the entire organ. The tissue was reticular, yet more compact than in the ox. In this instance, too, two types of cells could be distinguished in this tissue (Fig. 7). In the superficial parts the tissue was comparatively poor in cells, mainly cells with small and dense nuclei and containing little cytoplasm being encountered there. This layer contained only occasional cells with a larger, oval and obviously chromatin-poor nucleus. Deeper down in the hypendyma, i.e., closer to the posterior commissure, the abundance of cells in the tissue increased and mainly the last-mentioned cell type with large nuclei was encountered there.

Capillaries were encountered in abundance immediately under the ependyma, forming here and there dense plaits around the basal parts of the ependymal cells. The deeper parts of the hypendyma contained larger blood vessels.

In the horse, too, nerve fibres could be demonstrated in the hypendymal tissue, which did not penetrate as far as the ependymal tissue.

No formations whatsoever similar to the hypendymal ducts described for the ox could be demonstrated in any part of the S.C.O.

Pig

Ependyma. — The ependymal cells were found to be tall and columnar. Here and there the tissue was thrown in folds and formed crypts similar to those encountered in the ox. The *recessus mesocoelicus* was comparatively well-developed.

In regard to their internal structure, the ependymal cells resembled those of the animals described in the foregoing. Granular mass could be seen in the cytoplasm of all cells, and some cells showed a goblet-like distension of their apical part. The apical part of the cells protruded in club-shape over the surface of the organ. The basal cell processes were comparatively short.

On the surface of the organ a mass occurring in droplets was encountered, which was clearly granular in places. The plaits formed by the cilia of the ependymal cells penetrated from the surface of the organ into this mass.

Reissner's fibre did not show any distinct organic connection with the surface of the S.C.O. in the pig either. The fibre could be distinguished in the loose mass on the surface of the organ, similarly as with the other animals.

Capillaries from the blood vessel network of the hypendyma penetrated in places into the ependyma, running in the interstices of the ependymal cells. No nerve fibres could be demonstrated in the ependyma.

Hypendyma. — This tissue layer was well-developed. In it, too, one could distinguish two types of cells, namely, cells with a small and compact nucleus and containing little cytoplasm, and cells with a large, oval vesicular nucleus. No duct structures

like those observed in the ox could be demonstrated in the region of the hypendyma of the pig.

Like that of the other animals, the hypendyma was abundantly vascularized. The capillaries formed a dense network around the basal parts of the ependymal cells. Nerve fibres could be observed in the region of the hypendyma.

Dog

Ependyma. — The cells of the ependyma were found to be tall and columnar. Strong wrinkling of the ependyma was characteristic of the S.C.O. of the dog. The *recessus mesocoelicus* was well-developed, penetrating deeply dorsally.

The internal structure of the ependymal cells was similar to that observed in the animals already described. Granular mass was observed in the cytoplasm of almost all cells; this mass was particularly concentrated in the apical parts of the cells. The basal processes of the cells were short, yet clearly demonstrable.

The superficial cells bore a cilium protruding into the loose, granular mass covering the surface. In the region of the *recessus mesocoelicus* this mass had settled to a coherent, dense formation. In this mass Reissner's fibre seemed to have its origin.

The interstices of the ependymal cells contained capillaries here and there even quite profusely, whereas no nerve fibres could be demonstrated in this region.

Hypendyma. — This tissue layer was scanty and it seemed to be nearly absent in places. The cell tissue was reticular, with cell elements of two different types, similarly as in the other animals examined.

The hypendyma contained an abundance of blood vessels. They formed a dense network protruding into the ependyma around the basal parts of its cells. Some nerve fibres could be demonstrated in the region of this tissue layer.

Discussion

Several of the observations resulting from the present investigations and related in the foregoing are similar to those reported in the publications mentioned at the beginning of this section. However, also some new findings could be recorded.

The structure of the S.C.O. was essentially similar in all species of animals now examined. In all instances a superficial ependymal layer could be distinguished, composed of tall, columnar cells and a hypendymal layer of a reticular tissue. The most important inter-species difference concerning the ependyma is the folding of the superficial layer in cattle and dogs. The structure of the ependymal cells was different mainly in respect of the degree of development of the basal processes; their size appeared to be directly related with the thickness of the hypendymal tissue layer. The hypendymal cells appeared to be classifiable into two groups with all animals, resembling of the microglia and macroglia cells in the region of the rest part of the nervous system.

From a functional point of view some interesting observations were made in the present investigation. In all the animals, cytoplasmic, granular mass could be observed in the ependymal cells, and the same kind of mass was also encountered in the free space of the cerebral ventricle on the surface of the organ. No similar observations have been made before with domestic animals. Bauer-Jokl (1917) has found in domestic animals on the surface of the organ a homogeneous, not granulated mass, which she interprets to be a secretion. Kolmer (1921) has been able sometimes to find granular material in the ependymal cells of the dog's S.C.O., but he rejects the idea of its being a secretion. Krabbe (1925) on the other hand has found the cytoplasm in these cells to be homogeneous as a rule. Some cells of the organ have been found to be distended into goblet shape, and a similar observation is reported by Kolmer (1921), although the latter interprets the distensions as resulting from unsatisfactory fixation. Pesonen (1940 a) has made exactly the same observations on the S.C.O. of the guinea pig. He interprets the granular mass encountered both in the ependymal cells and on the surface of the organ, as well as the goblet-shaped cells of the ependyma, as an indication of secretory activity. It is indeed difficult to find any other explanation for them. Pesonen's results of investigation as well as the observations made in the present work speak against Kolmer's opinion, according to which the goblet-shaped distensions of the cells would have been called into existence by inadequate fixation.

The overall structure of the S.C.O. of the guinea pig, which

is essentially similar to that of the S.C.O. of the animals now examined, is considered by Pesonen as further indication that a tubular gland is concerned. The duct-like structures encountered in the S.C.O. of the ox can be said to carry even stronger resemblance to a typical glandular structure, their similarity to the ducts of tubular glands occurring elsewhere in the organism being rather obvious. No mention can be found in the previous literature of these formations. The only statements indicative of such findings are possibly those by Krabbe (1925) and Turkewitsch (1936 a, 1936 b) of the compact cell islets composed of cells greatly resembling those of the ependyma and encountered in the hypendyma. However, no duct-like structures were observed in them. Turkewitsch has found formations in the S.C.O. of the bovine foetus, which he designates as cylindrical ducts, but the description given of them does not correspond to the appearance of the ducts found in the present work. Describing them, Turkewitsch says: »Diese Gänge haben, ebenso wie die übrigen Höhlen des Hypendyms keine spezielle sie innen bekleidende Zellschicht» (These ducts, like all other cavities in the hypendyma, have no special cell layer lining them internally). It is further stated that »ins Innere dieser Gänge Blutgefässe eindringen» (blood vessels penetrate into the interior of these ducts). These descriptions do not fit the duct structures found in the adult animal in the present investigation. The author also states that the ducts observed in the foetus in his investigation are not encountered in the adult.

The hypendymal ducts are probably no immediate continuations of the crypts into the region of the hypendyma, since the basal part of the crypts could be noted to be distended, sac-like. Moreover, such ducts were also encountered in areas where the ependyma was smooth and they could be noted to open onto the surface of the organ even in the area of this kind of ependyma. Also that fact speaks in favour of their independent nature that the ducts penetrate even quite deeply into the tissue of the posterior commissure.

The observational finding relating to the parietal cells of the ducts, i.e., the occurrence of granular mass in their cytoplasm suggests that similar secretory activity would take place in the ducts, too, as that which can be observed in the ependymal cells. The finding of granular mass also in the lumen of the ducts can

be construed to indicate that the ducts discharge their secretion directly onto the surface of the organ. The capillary spaces between the parietal cells, which were full of granular mass, are obviously comparable to intercellular secretory canaliculi.

Earlier investigators of the S.C.O. (e.g. Pesonen 1940 a) have called attention to the profuse vasculature of the organ and considered it indicative of a lively metabolism. This observation could also be corroborated in the S.C.O. of the domestic animals. Another interesting fact was the close connection between the basal processes of the ependymal cells and the subependymal network of capillaries. This has not been mentioned by any one of the earlier authors who have studied the S.C.O. of domestic animals.

No actual attachment of Reissner's fibre to the surface of the S.C.O. could be noted in the present work. A connection of this kind has earlier been reported particularly by Agduhr (1922) who was able to show that in the ox the fibrils of Reissner's fibre indeed even penetrated into the interstices of the ependymal cells. In the present work the sole connection between Reissner's fibre and the surface of the S.C.O. was noted to consist of a network made up of delicate fascicles formed by the mass encountered on the surface and showing, in places, a distinct granular structure. Neither could the observation of Nicholls (1912) and Jordan (1925) be corroborated, according to which Reissner's fibre would be an immediate continuation of the cilia of the ependymal cells. However, the matted cilia could be noted to intermingle with the above-mentioned granular mass that constitutes the contact medium between Reissner's fibre and the surface of the organ. On the strength of the observations made in this work it would seem obvious that Reissner's fibre indeed originates just in the loose, granular mass encountered on the surface of the organ, which is made up of granular material similar to that encountered in the cytoplasm of the ependymal cells.

Synopsis

For the reason that discrepancies exist in the pertinent literature, an investigation of the subcommissural organ of ox, horse, pig and dog was undertaken, employing the procedures used in

previous investigations and aiming at a clarification of the microscopic structure of the organ. Particularly such properties were made the subject of study which could be thought to have significance in regard to the assessment of the functional character of the organ. In all the animals examined an outer ependymal layer could be distinguished in the organ, made up of tall, columnar cells, and a hypendymal layer, which presented itself as a reticular tissue made up of cells of the microglia and macroglia cell type. In the ependymal cells cytoplasmic, granular mass was encountered in all the animals. Similar mass was also observed in the ox in the parietal cells of the duct structures found in the hypendyma, as well as freely in the cerebral ventricle on the surface of the organ in all the animals examined and in the lumen of the above-mentioned ducts of the ox. Some cells of the ependyma could be noted to be distended to goblet-like shape. Profuse vasculature was observed in the subcommissural organ, which fact can be considered an indication of lively metabolism. These findings, together with the structure of the organ resembling that of a tubular gland, are thought to speak in favour of glandular activity occurring in the organ. Reissner's fibre was not directly attached to the subcommissural organ. The fibre obviously originates in the loose granular mass on the free surface of the ependyma, into which the matted cilia of cells penetrate from the surface of the organ.

3. THE LOCALIZATION OF THE SELECTIVELY STAINING »SECRETORY MATERIAL» IN THE SUBCOMMISSURAL ORGAN

With a great number of different animal species it has been possible to demonstrate in the ganglion cells of the hypothalamic supraoptic nucleus and paraventricular nucleus selectively staining granules or droplets in their cytoplasm and these particles are thought to be a secretion of the said cells (Scharrer and Scharrer 1954). This secretion, the so-called neurosecretory material can be demonstrated by means of certain staining methods, the most important ones being the chrome-alum-haematoxylin-phloxine method (Gomori 1941 a), the aldehyde-fuchsin method (Gomori 1950), and the phosphotungstic acid-Congo red method (Matsuura 1925). The present conception of neurosecretion builds up mainly on results obtained with the aid of these procedures.

The S.C.O. has also been investigated by these methods and the ependymal cells in its region have been found to contain »secretory material» reacting positively to the stains. Previously examined species of animals are: dog and cat (Bargmann and Schiebler 1952), rat, mouse, guinea pig and hamster (Wislocki and Leduc 1952 b, 1954), lower vertebrates (Mazzi 1952, 1954), *Anguilla vulgaris* (Stutinsky 1953), the chicken embryo (Wingstrand 1953, Grignon 1956), various animal species from *Cyclostoma* to man (Oksche 1954, 1956), *Salmo salar* (Arvy, Fontaine and Gabe 1955), frog (Okada 1955), tortoise (Fleischhauer 1957), *Gecko japonicus* (Murakami, Ban and Aiura 1957), and the human foetus (Wislocki and Roth 1958). The majority of the said investigations have been made by means of the chrome-alum-haematoxylin-phloxine method. In the investigations of Wislocki and Leduc (1954) and of Wislocki and Roth (1958) moreover the aldehyde-fuchsin method has been employed, while

Murakami, Ban and Aiura (1957) have used the aldehyde-fuchsin method alone.

Concerning the animals included in the material of the present series of investigations no detailed description has been given so far of their S.C.O. employing the above-mentioned conventional methods, excepting the dog, which also has been studied merely by means of the chrome-alum-haematoxylin-phloxine method. For this reason it was thought appropriate to carry out an investigation with these animals using all three staining methods.

Methods

The material was fixed either in 10 % formalin or in Bouin's fluid. Upon fixing, the material was embedded in paraffin in the conventional manner and sections were made at 3 to 10 micra in the sagittal, horizontal and frontal planes.

The chrome-alum-haematoxylin-phloxine method was used in its original form as presented by Gomori (1941 a).

The aldehyde-fuchsin procedure employed was the modification of Gomori's (1950) method presented by Landing, Hall and West (1956). The counterstain applied to part of the material was performed with orange G, light green SF or haematoxylin.

The phosphotungstic acid-Congo red method was used as modified by Sloper (1955) from Matsuura's (1925) original method.

Results

All staining methods employed in this instance proved to possess the same degree of selectivity. With one and the same animal species completely identical localizations of the stainable material were obtained with each particular stain. The material acquired a deep blue stain with chrome-alum-haematoxylin-phloxine, a violet stain with aldehyde-fuchsin, and a bluish stain with phosphotungstic acid-Congo red. The localization of the stainable material was somewhat different with the various animal species.

Ox. — A comparatively great quantity of selectively stainable material was established, occurring mainly in two locations (Figs. 8 and 9). In the tall, columnar cells of the ependyma the

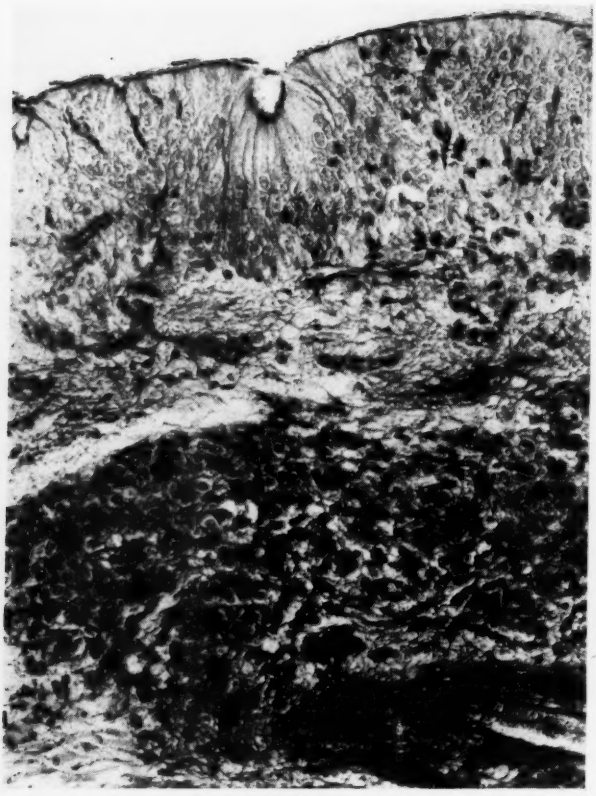

Fig. 8. Ox. — Part of longitudinal section of the S.C.O. In the hypendyma numerous ducts full of selectively staining material. Below, the posterior commissure. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 240 ×.

material occurred as a finely divided, granular mass, which coloured the entire cell layer when inspected under low magnification. The granules were located in the cytoplasm and they were most abundant in the apical part of the cell. As a rule a continuous zone of granules could be encountered at the apical limit of the cell (Fig. 10). However, copious accumulations of granules could also be noted in places in the basal part of the ependymal cells and also in the basal cell processes enveloping the capillaries. Similar granular material occurred on the surface of the ependymal cells in the free space of the cerebral ventricle.

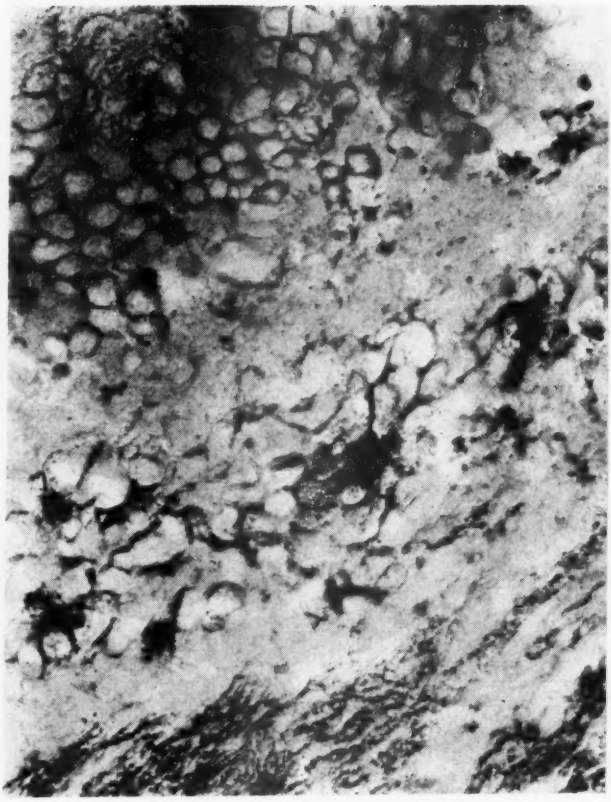

Fig. 9. Ox. — Portion of the hypendyma with ducts. »Secretory material» in the ducts. The ependyma visible in the upper part of the photograph. Below, the posterior commissure. — Aldehyde-fuchsin without counterstain. Magnification ca. 600 ×.

The other main localization of the material were the duct-like structures in the hypendymal region (Figs. 8, 9, 11, 12, 13, 14, and 15). The conical cells limiting the ducts contained an abundance of cytoplasmic, stainable granular material, which was most abundant in the apical parts of the cells adjacent to the lumen of the duct. The capillary spaces between the cells were filled with similar granular material, which also filled the lumen of the duct. In the lumina the quantity of this material was very plentiful as a rule. No stainable material could be established in any other region of the hypendyma.

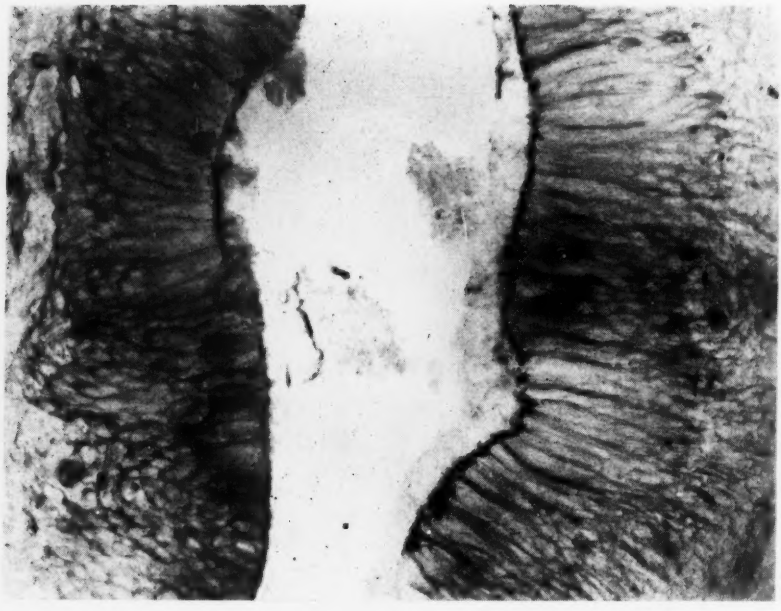

Fig. 10. Ox. — »Secretory material» in the ependyma, particularly in the apical parts of the cells. — Aldehyde-fuchsin without counterstain. Magnification ca. 300 ×.

In some places in the loose, granular mass on the surface of the organ denser fascicles of uniform thickness and of varying length, apparently fragments of Reissner's fibre, could be distinguished (Fig. 16). In places these fragments were connected with the surface by the granular mass.

Horse. — »Secretory material» was observed in the tall ependymal cells of the S.C.O. and on the surface of the organ in the free space of the cerebral ventricle.

In the ependymal cells the granular mass was located in the cytoplasm. It was fairly uniformly distributed all over the cytoplasm, although in numerous places the material formed a continuous, narrow zone of granules in the apical part of the cell closest to cell limit. The basal processes of the cells protruding into the hypendyma, too, contained such material abundantly in places, where it formed accumulations (Figs. 17 and 18).

Like in the ox, there was loose granular mass on the surface of the organ in the horse. In some sections similar, strongly

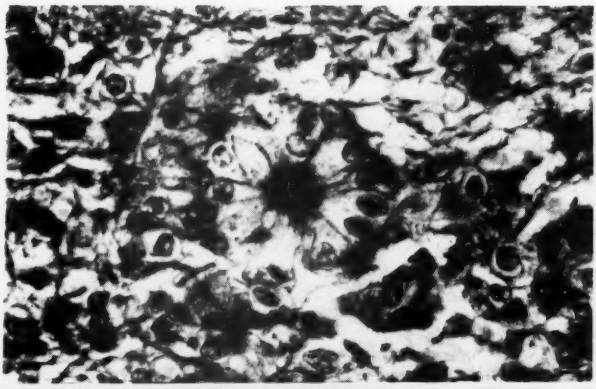

Fig. 11.

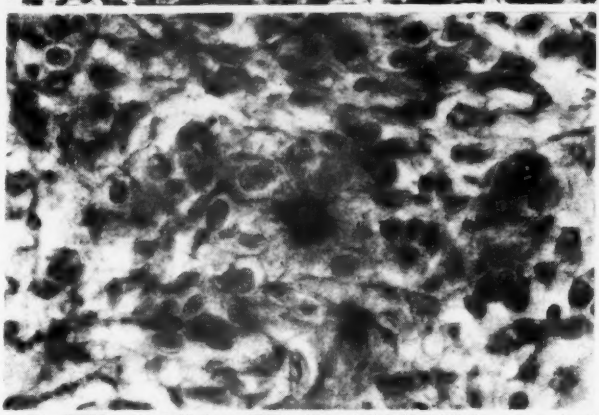

Fig. 12.

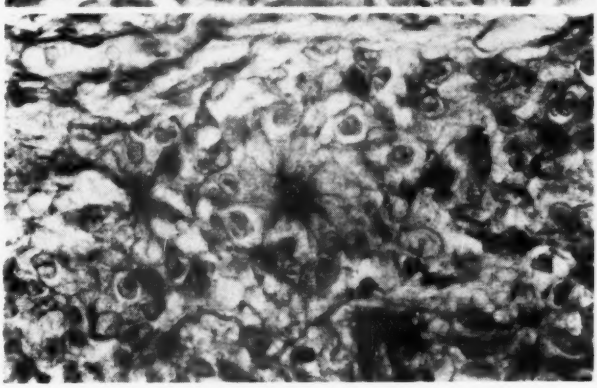

Fig. 13.

Figs. 11 — 13. Ox. — Hypendymal ducts. — Fig. 11. Chrome-alum-haematoxylin-phloxine. Magnification ca. 600 ×. Fig. 12. Aldehyde-fuchsin + haematoxylin counterstain. Magnification ca. 500 ×. Fig. 13. Phospotungstic acid-Congo red. Magnification ca. 600 ×.

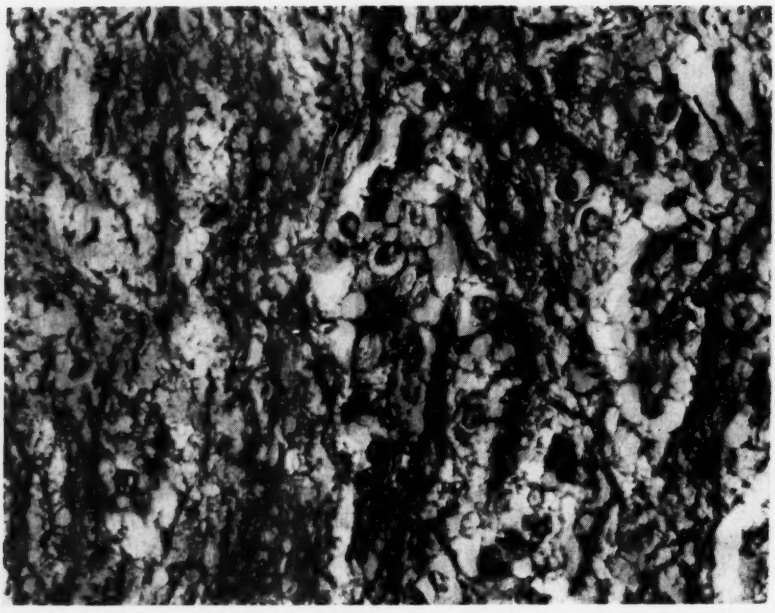

Fig. 14. Ox. — Hypendymal duct with »secretory material» deep between the fibres of the posterior commissure. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 500 ×.

staining fragments of Reissner's fibre could be noted in this loose mass (Fig. 19) as were reported in the ox.

In the region of the hypendyma material occurred in its superficial parts in the above-mentioned basal processes of the ependymal cells only. In the deeper layer, again, finely divided granular material was found in the cytoplasm of some macroglia-like cells. Such cells occurred also occasionally in the hypendymal tissue interpenetrating the fibres of the posterior commissure.

Pig. — Material reacting positively to the stain occurred only in the ependymal cells. As in the other animals, the material in the cytoplasm occurred in the form of finely divided granules (Figs. 20 and 21). The granules were most abundant at the apical boundary of the cells and in their basal processes. The material on the surface consisted of a granular, loose mass, in which short, compact fragments of Reissner's fibre could be observed here and there. The ependymal capillaries were surrounded in places by accumulations of granules.

Fig. 15. Ox. — Point of opening of a hypendymal duct in the ependyma. Below this point, a cross section of a similar duct, indicated by arrows. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 660 ×.

Dog. — The positively reacting material in the ependymal cells occurred as a finely divided, granular mass, which was particularly located at the apical boundary of the cells, where it formed a comparatively thick layer (Figs. 22 and 23). Elsewhere in the region of the cytoplasm the material occurred scantily except in the basal processes, where even quite abundant granular accumulations occurred here and there. In some places around the walls of blood vessels in the ependymal region small accumulations of granules could be established. Similar accumulations seemed to occur elsewhere, too, in the interstices of the ependymal cells.

On the surface of the organ similar granular material was

Fig. 16. Ox. — Portion of the ependyma. Part of Reissner's fibre visible on the surface. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 500 ×.

found, in which compact fragments of Reissner's fibre could be demonstrated in some sections.

In the region of the scanty hypendyma even quite abundant accumulations of granules could be observed in places; as a rule they appeared to be connected with the basal processes of the ependymal cells penetrating into the hypendyma. Also around the hypendymal blood vessels such granular accumulations occurred here and there.

Discussion

The three methods employed in the present work have proved to be well suitable for establishing the neurosecretory material

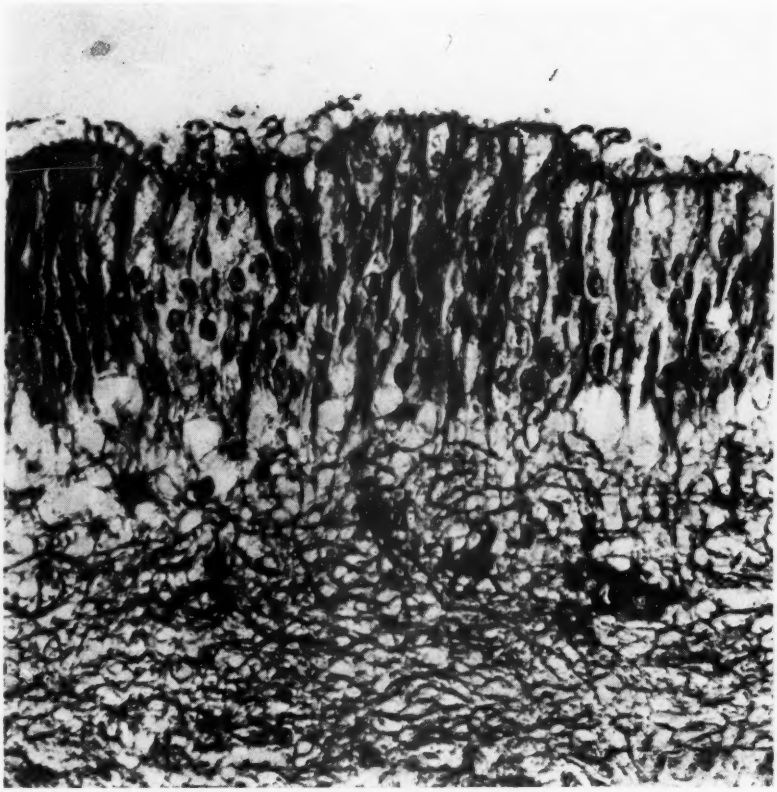

Fig. 17. Horse. — »Secretory material» in the ependyma. In the hypendyma, accumulations of material in contact with the basal processes of the ependymal cells. — Aldehyde-fuchsin + Heidenhain's iron-haematoxylin counterstain. Magnification ca. 500 ×.

in the hypothalamo-hypophysial system (Bargmann 1949, Dawson 1953, Sloper 1955). No one of these methods is a specific method that can be employed to stain some substance with a definite chemical composition; they are merely selective. An entirely empirical approach has shown them to be applicable in demonstrating selectively the hypothalamo-hypophysial neurosecretion. The observations made in the present investigation tend to indicate that also in the S.C.O. of the animals examined all these methods stain selectively a type of substance, the localization and distribution of which furnishes good reason for the assumption that it has the character of a secretion of the organ.

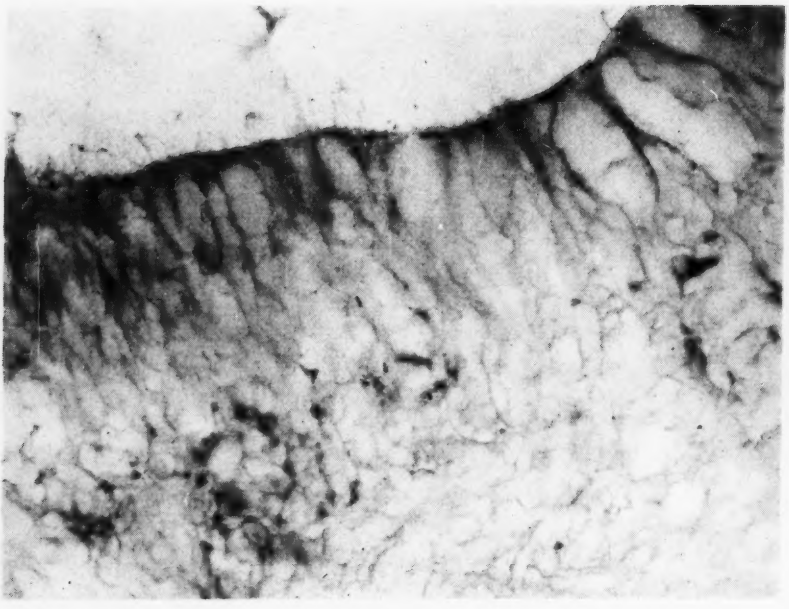

Fig. 18. Horse. — »Secretory material» in the ependymal cells, particularly in their apical parts and in the cytoplasmic processes. — Aldehyde-fuchsin without counterstain. Magnification ca. 400 ×.

In regard to the character of the stainable material in the hypothalamo-hypophysial system very impressive arguments have been presented to the effect that it would contain the hormones of the posterior pituitary or their carrier substance (e.g. Hild and Zetler 1951, 1952). However, since as a rule the methods display no specificity, it is not possible from this to draw the inference that in the region of the S.C.O., too, the substance discovered in this way would have the same character. It should also be noted that a very great variety of structures can be stained by these methods (e.g. Halmi and Davies 1953); therefore no conclusions relating to the composition of the material are possible.

Regardless of what may be the chemical nature of the stainable material, its existence and distribution in the region of the organ with the different species of animals supports the conception that the organ would have a secretory function. This applies equally to the cytoplasmic material in the ependymal cells of

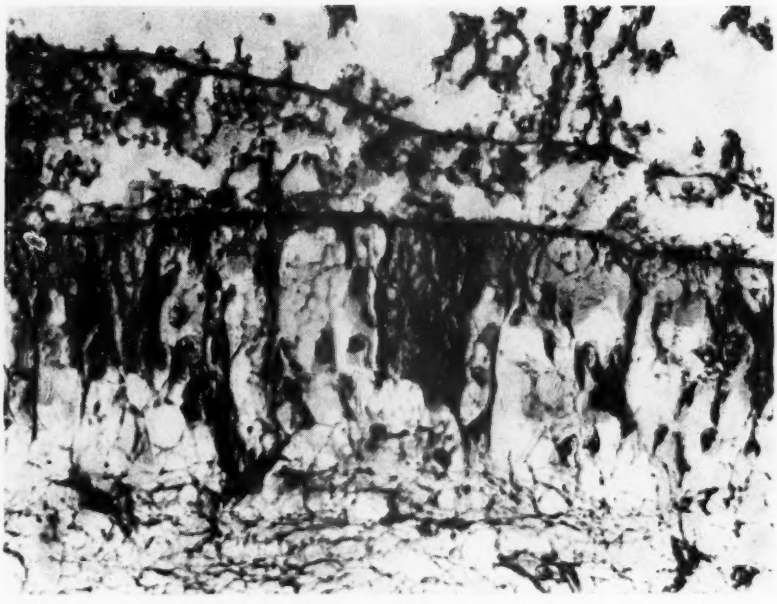

Fig. 19. Horse. — Section of the marginal part of the S.C.O., showing the transition of its ependyma into usual ependyma. On the surface »secretory material», containing a portion of Reissner's fibre. — Aldehyde-fuchsin + Heidenhain's iron-haematoxylin counterstain. Magnification ca. 400 ×.

all animal species and to that in the parietal cells of the hypendymal ducts observed in the ox.

In their investigations, Oksche (1954, 1956) and Grignon (1956) have made observations from which they draw the inference that the tall ependymal surface cells are bipolar in their potential secretory activity, i.e., the secretion from the cells is discharged both onto the free surface of the organ into the cerebral ventricle and via the basal cell processes directly into circulation. These observations are based on the distribution of the secretory material particularly in the apical parts of the cells and in their basal pole. Moreover, Oksche has found variations between the animal species in that lower animals display much more remarkable basal accumulations of the material than species in a higher stage of evolution. In conformity with this, Murakami, Ban and Aiura (1957) have observed with the *Gecko* an accumulation of the material in great quantities around the capillaries;

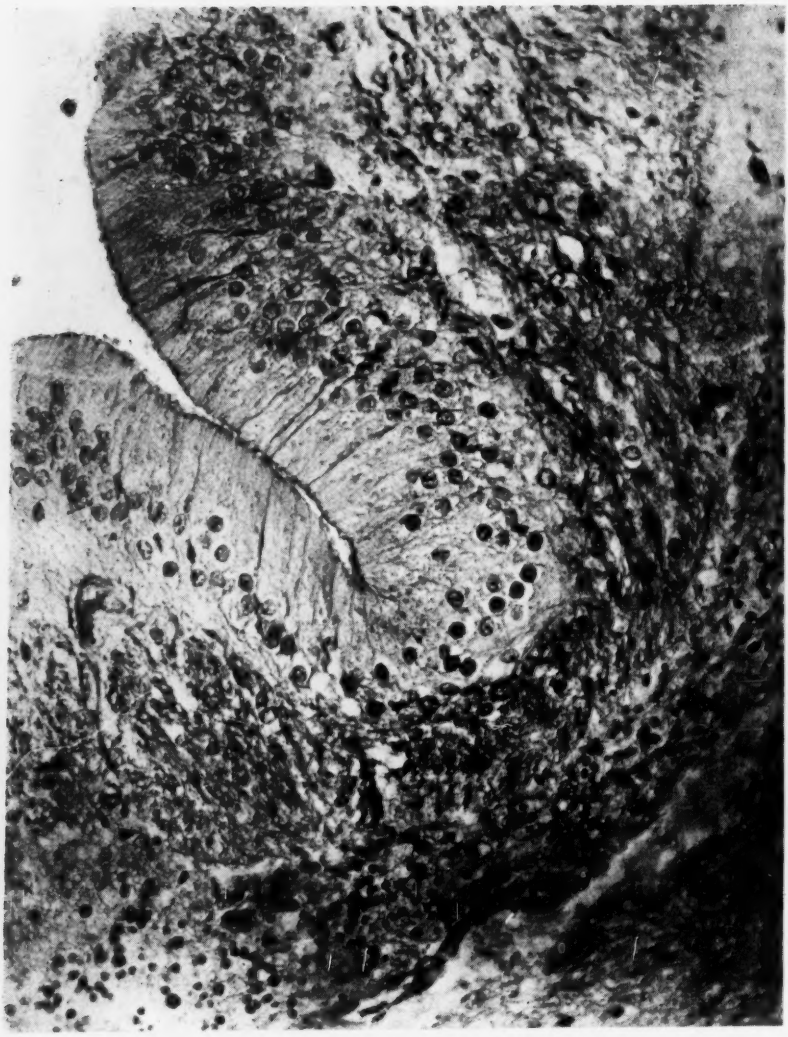

Fig. 20. Pig. — Portion of the S.C.O. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 300 ×.

according to the authors this, too, is indicative of the possible discharge of secretion directly into the circulation. In the present work observations leading to similar conclusions could be made in all the species examined. In particular with horse and dog but also with the other animals there could distinctly be observed

Fig. 21. Pig. — Portion of the ependyma, with cells containing selectively stained granular material. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 660 ×.

an accumulation of the material in small aggregations in the basal cytoplasmic processes of the ependymal cells; here and there such material occurred even in very considerable quantities around the blood vessels. Also on the surface and in the apical parts of the cells a distinct tendency towards an accumulation of granules was present in all animals. These observations may

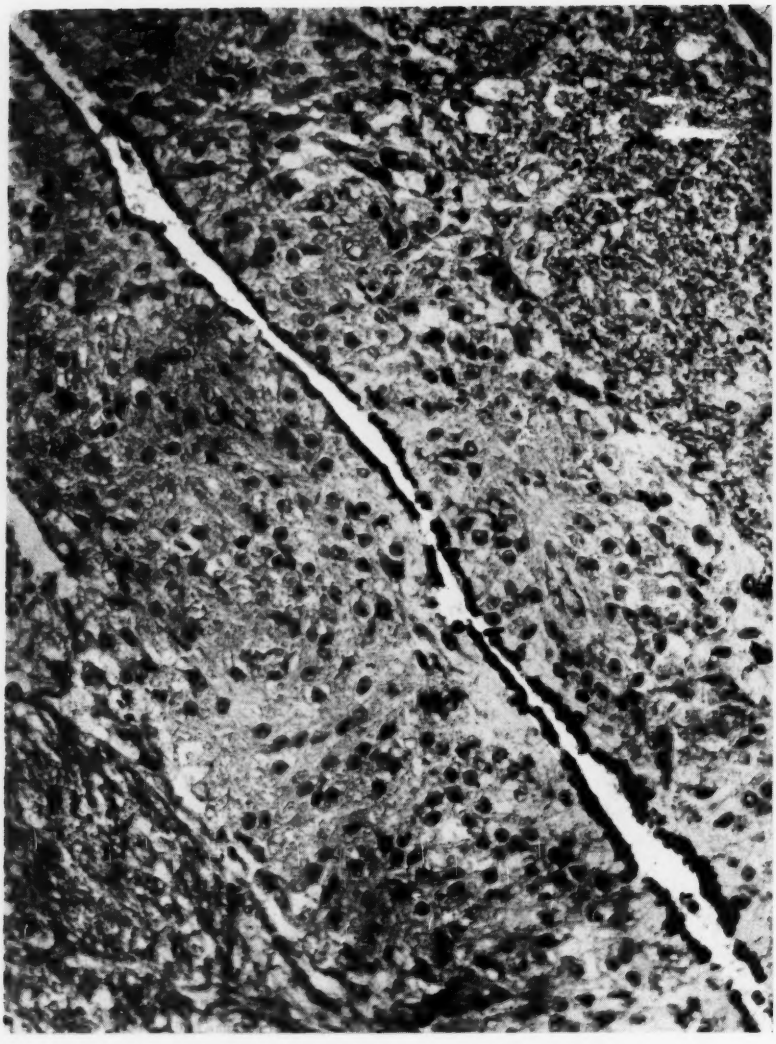

Fig. 22. Dog. — Longitudinal section of a fold formed by the ependyma. »Secretory material» particularly in the apical parts of the cells. — Chrome-alum-haematoxylin-phloxine. Magnification ca. 480 ×.

speak in favour of the opinion that in the domestic animals, too, secretion would occur in two directions, i.e., in part directly into the cerebral ventricle and in part into the basal capillary network of the ependyma, in the manner of the secretion taking place in

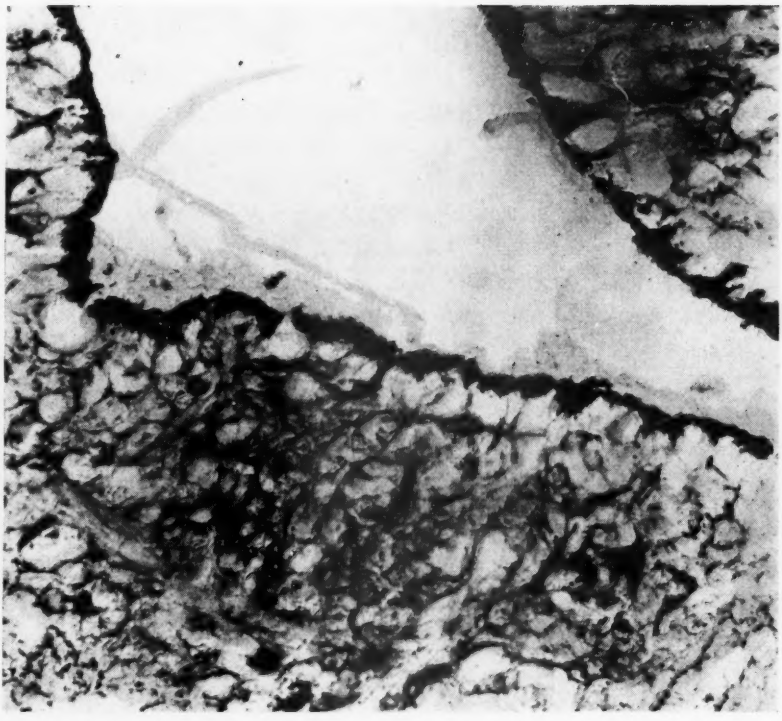

Fig. 23. Dog. — »Secretory material» in the cytoplasm of the ependymal cells. — Aldehyde-fuchsin without counterstain. Magnification ca. 660 ×.

endocrine glands. In the region of the posterior hypophysis, several investigators have interpreted the neurosecretory material around the blood vessels as indicating secretion into the circulation (e.g. Hanström 1952). It is evident that on the same grounds secretion could be thought to occur directly into the blood stream in the S.C.O. as well.

It was not possible to make any direct observations indicative of a transport of the »secretory material» via the posterior commissure into the subarachnoidal space, similar to those made by Okada (1955) with the frog and by Murakami, Ban and Aiura (1957) with the *Gecko*. The sole indication suggestive of this were the hypendymal ducts encountered deep in the tissue of the posterior commissure of the ox.

In the hypendyma of the horse finely divided granulation reacting positively to the stain was encountered in the cytoplasm of the macroglia-like cells. It is hard to make any positive statement with regard to its nature. The said granules seem to have no connection with the ependymal cell tissue on the surface nor with the capillary network. One might assume that the cells belong to a similar, possibly more extensive subependymal system as that observed by Wislocki and Leduc (1952 b) in small rodents, especially in the guinea pig. The authors say in regard to these cells that »their granular material does not necessitate the assumption of any direct connection between these granules and the similarly stained reactive material in the cells of the subcommissural organ». In the other animals no such cells were encountered. This might indicate a different quality of the subependymal tissue of the horse on the whole but no studies have been made of this problem.

In the reactive granular mass on the free surface of the organ, in the cerebral ventricle, dense fragments of fibres could be observed in all animals, possessing similar staining properties as the granular material found in the cells of the organ and on its free surface. These fibre formations were probably pieces that had resulted on sectioning from the initial part of Reissner's fibre. No solid connection could be established between this fibre and the surface of the S.C.O. It appeared as if the fibre originated in the loose granular mass on the surface of the organ. Similar observations have been made earlier by some authors (e.g. Wislocki and Leduc 1952 b, Mazzi 1952, Wingstrand 1953) in their investigations with various animals; they have proposed the idea that Reissner's fibre is formed of the secretion of the S.C.O. This seems to be borne out by the similar staining properties of the said »secretion» and the fibre in the examined domestic animals as well.

Of the animal species included in the present material of investigation, only the dog has been subject to a corresponding study of the distribution of the selectively stained »secretory material» in the S.C.O. Bargmann and Schiebler (1952) have made similar observations with the dog as those reported here. However, they found in the region of the hypendyma granular accumulations which have remained problematic in regard to their

nature. In the findings of the present work the granular accumulations of the hypendyma were connected with the basal processes of the ependymal cells as a rule.

Synopsis

Selectively staining material was found by all the procedures employed in the subcommissural organ of the species of animals examined. Its localization was somewhat variable but it was encountered in all animals in the cytoplasm of the ependymal cells and on the surface of the organ in the free space of the cerebral ventricle. The ox exhibited this kind of material also in the hypendymal duct structures in the cytoplasm of the cells lining their walls and in the lumen of the ducts. Similar material also occurred in the cytoplasm of the macroglia-like cells in the hypendyma of the horse. The existence of this material and its distribution in the ependymal cells and in those of the duct structures in the ox are thought to speak in favour of possible secretory activity. The distribution of the material contained in the ependymal cells is thought to be indicative of the potential secretion occurring in a bipolar manner, i.e., partly into the cerebral ventricle and partly into the system of blood vessels. The dense fibre formations in the granular mass on the free surface of the organ were obviously fragments produced by the sectioning of Reissner's fibre, having the same staining properties as the »secretion» of the organ.

4. ON THE DISTRIBUTION OF SOME ENZYMES IN THE SUBCOMMISSURAL ORGAN

Numerous histochemical methods are now available for the demonstration and location of enzymes in tissues. A great variety of different tissues have been investigated with their aid, among them various parts of the central nervous system. The results from such investigations have been used in attempts at inference with regard to the metabolic character of the tissues, the knowledge of which is an essential prerequisite for the determination of their functions.

The S.C.O., too, has been investigated before by means of these histochemical methods although to a fairly small extent. According to the available literature, histochemically demonstrable activity of acid phosphatase and non-specific esterases could be established in the S.C.O. of rat, guinea pig, rabbit and hamster (Leduc and Wislocki 1952) and in the S.C.O. of the rabbit furthermore activity of succinic dehydrogenase (Shimizu, Morikawa and Ishi 1957).

The purpose of the present investigation included the histochemical study of the acid and alkaline phosphatase, succinic dehydrogenase and non-specific esterase activities in the S.C.O. of ox, horse, pig and dog and of their distribution in the said organ of these animals.

Methods

Acid phosphatase. — For the determination of the enzymatic activity and for its localization, Eränkö's (1951 a) modification of Gomori's (1941 b) technique was used. The material was fixed in 4 % formalin neutralized over calcium carbonate during two

hours at room temperature prior to incubation and it was sectioned with the freezing microtome at 10 to 30 micra. The pH value of the substrate mixture was 4.7, the incubation time 15, 30, 45 minutes, etc. in an increasing series up to three hours. Incubation took place at 37° C.

The specificity of the reaction was checked by incubation in a mixture from which the glycerophosphate had been omitted, as well as by adding M/200 sodium fluoride to the standard substrate mixture (Eränkö 1952).

Alkaline phosphatase. — For demonstration and localization, Gomori's (1939) method was used in the manner described by Lillie (1954). Fixing was carried out in 80 % ethyl alcohol during 24 hours at room temperature. Upon embedding in paraffin the material was sectioned at 5 to 10 micra, attached on slides and deparaffinized. Incubation times of 30 minutes to two hours were used.

Specificity was checked either by omitting the glycerophosphate from the substrate mixture or by heating the sections to 80° C during 10 minutes, prior to incubation (Newman, Kabat and Wolf 1950).

Succinic dehydrogenase. — The method of Seligman and Rutenburg (1951) was used but instead of ditetrazolium chloride, as provided by the authentic method, neotetrazolium chloride (Nutritional Biochemicals Corporation) was used as a staining agent (Shelton and Schneider 1952). The fresh tissue was cooled in the freezing microtome and sectioned at 20 to 40 micra. Incubation took place for two hours at 37° C and the substrate mixture had a pH of 7.6. Upon incubation the sections were treated in 10 % formalin for 30 minutes.

In order to check the specificity, 0.37 % sodium malonate (Seligman and Rutenburg 1951) was used as an addition to the incubation mixture, or the sodium succinate was omitted.

Non-specific esterases. — The method developed by Nachlas and Seligman (1949) was used. The material was fixed in acetone at 4° C during 24 hours and sectioned at 6 micra upon embedding in paraffin. The staining agent was Diazo Blue B (Edward Gurr, Ltd.) and the substrate α-naphthyl acetate. Incubation took place at room temperature for 20 minutes. The substrate mixture had a pH of 7.8.

The potential effect of cholinesterases was inhibited by means of eserine sulphate at 10^{-5} M concentration and the lipases were blocked with 0.05 M quinine hydrochloride (Nachlas and Seligman 1949).

In the determinations of all enzymes part of the preparations were counterstained with appropriate staining agents as described by Lillie (1954), in order to facilitate localization of the activity.

Results

All the observations reported in the following were made in instances where the enzymatic activity was manifestly specific on the strength of the result recorded in the specificity tests.

Acid phosphatase. — Darkening of the nuclei could be noted as early as after 15 minutes in the ependymal cells of all the animals and in the parietal cells of the hypendymal ducts of the ox. More prolonged incubation resulted in an increasingly darker shading and after two hours' incubation the nuclei in the said cells were already completely opaque. No remarkable differences could be noted between the different species of animals or between the various parts of the organ. In the same cells a fine precipitation could be noted in the cytoplasm already after 15 minutes' incubation. This reaction, too, increased slightly with increasing incubation time (Figs. 24 and 25).

In the glia-like hypendymal cells darkening of the nuclei was observable after one hour's incubation (Figs. 24 and 26), whereas no cytoplasmic reaction could be noted even after three hours' incubation. In the walls of the hypendymal blood vessels no reaction could be established.

Alkaline phosphatase. — The ependymal cells revealed activity as early as after 30 minutes' incubation in all animals. This was also the finding with regard to the parietal cells of the ox's hypendymal ducts (Fig. 26). The dark shading was most clearly evident in the cytoplasm although the nuclei reacted, too. After one hour's incubation the darkening attained such strength in some cells that they became nearly opaque.

The glia-like cells of the hypendyma displayed no darkening even with two hours' incubation. However, an exceedingly distinct

Fig. 24. Ox. — Acid phosphatase reaction in the S.C.O. in the region of the *recessus mesocoelicus*. — Gomori's method. Frozen section from an organ fixed in 4 % formalin. Incubation time 60 minutes. No counterstain. Magnification ca. 200 ×.

reaction was observed in the walls of the hypendymal blood vessels already with an incubation of 30 minutes. A similar reaction could also be noted in the region of the surrounding brain tissue in the walls of the blood vessels, while the true brain tissue did not react.

Succinic dehydrogenase. — All the animals examined exhibited activity of this type in the region of the S.C.O. The reaction was present in the ependymal cells of all the animals and in the ox moreover in the parietal cells of the hypendymal ducts. The cytoplasm of the cells acquired a bright red stain, and it contained numerous small, dark-blue diformazan granules (Fig. 27). No differences worth mentioning could be noted between the ependymal cells, but the largest granules were formed in the parietal cells of the hypendymal ducts of the ox. The nucleus did not react in any cell. The walls of the blood vessels exhibited blue granular mass here and there but the true hypendymal tissue did not react.

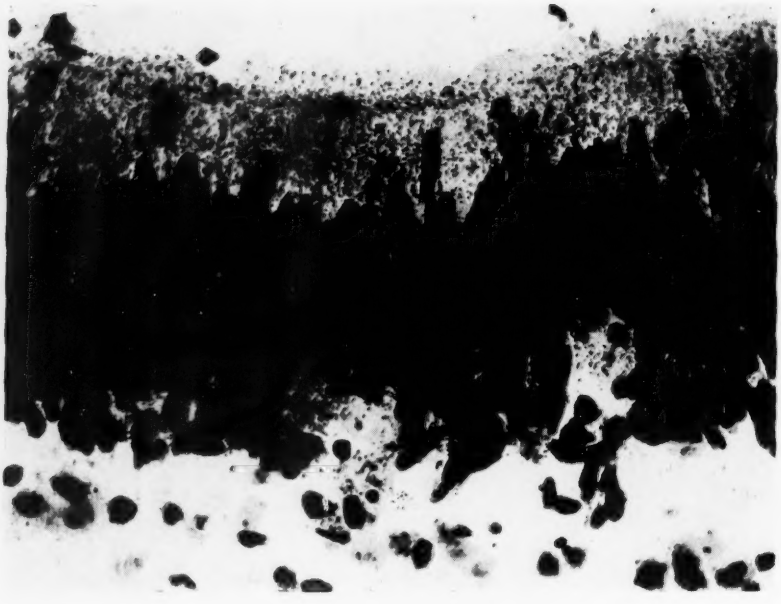

Fig. 25. Horse. — Intense acid phosphatase reaction in the nuclei of the ependymal cells. Fine precipitation also in the cytoplasm. In the hypendyma, a reaction in the nuclei. — Gomori's method. Frozen section from an organ fixed in 4 % formalin. Incubation time 90 minutes. No counterstain. Magnification ca. 600 ×.

The posterior commissure was not always stained in freshly prepared preparations but it did turn light red if it was left in storage for a few days.

Non-specific esterases. — Positive reaction could be noted in the ependymal cells of all the animals and in the ox also in the parietal cells of the hypendymal ducts (Fig. 28). The reaction manifested itself as a blue colour in the cytoplasm of the cells. On the other hand, a slight colour could be observed in the walls of the blood vessels.

Discussion

According to the results obtained, histochemically demonstrable enzymatic activity of acid and alkaline phosphatase, succinic dehydrogenase and non-specific esterases could be established in the S.C.O. of the investigated animal species. The activity

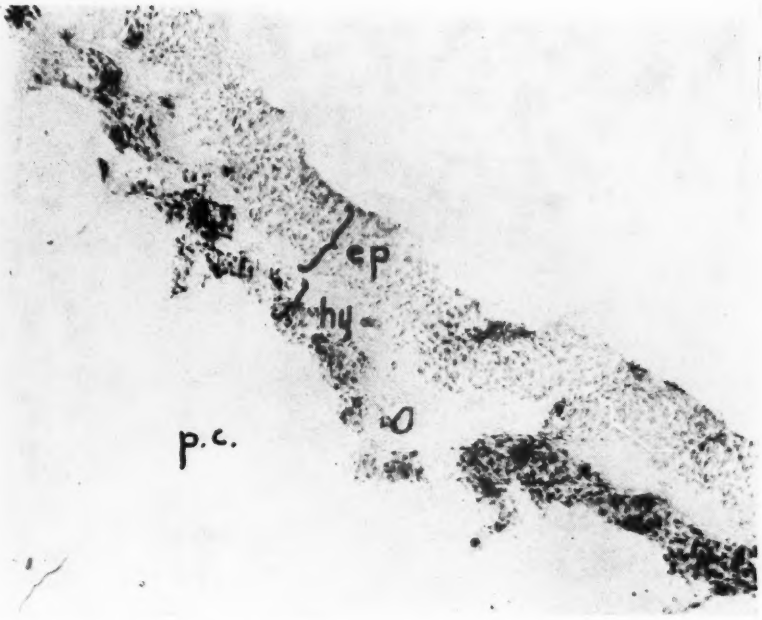

Fig. 26. Ox. — Alkaline phosphatase reaction in the ependymal cells and in the parietal cells of the hypendymal ducts. Reaction also observable in the walls of blood vessels. ep = ependyma, hy = hypendyma, p.c. = posterior commissure. — Gomori's method. Incubation time 30 minutes. No counterstain. Magnification ca. 260 ×.

was distinct in the ependymal cells and in the ox furthermore in the parietal cells of the hypendymal ducts. The glia-like cell tissue of the hypendyma showed activity of acid phosphatase only, with the comparatively short incubation times employed. Even for this enzyme the reaction was limited to the nuclei.

On assessment of the results one should bear in mind the shortcomings in histochemical techniques displayed even by the most far-developed methods for determining the activity of enzymes and its localization in tissue. The most notable sources of error have been, first of all, the changes occurring during the time interval between the death of the animal and the fixing or freezing of the material; secondly, the fact that diffusion of the enzymes particularly during incubation is conducive to an incorrect picture of their localization. Quite obviously these factors could not be eliminated in the present work either although the

Fig. 27. Ox. — Diformazan granules in the ependymal cells and in the parietal cells of the hypendymal ducts. ep = ependyma, hy = hypendyma. — Seligman-Rutenburg's method for the demonstration of succinic dehydrogenase. Incubation time 120 minutes. Magnification ca. 600 ×.

best attempt has been made to minimize their influence. The material from the animals to be slaughtered was usually received for further treatment no earlier than about 30 minutes after the animal's death. In the case of a few calves and dogs the material could be frozen or immersed in the fixing fluid about 1 to 3 minutes after disruption of their circulation, this being achieved with the aid of narcosis. However, no fundamental diversity in results could be noted on comparison of the material from these animals with the remaining material. Since diffusion may possibly have quite a notable influence, the attempt was made to minimize it by using the shortest possible incubation time. In regard to acid

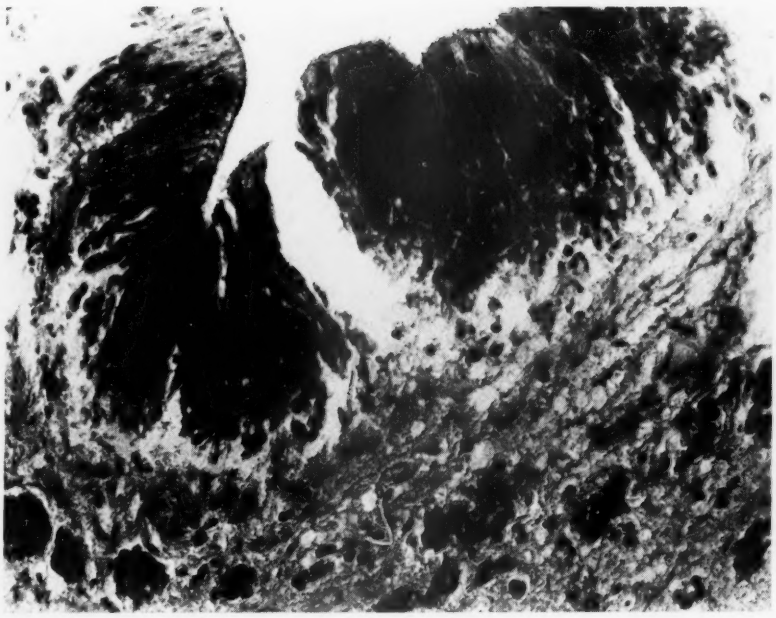

Fig. 28. Ox. — Reaction of non-specific esterases in the ependymal cells and in the parietal cells of the hypendymal ducts. — Nachlas-Seligman's method + haematoxylin counterstain. Magnification ca. 400 ×.

phosphatase a satisfactory result can be obtained by Eränkö's modification (Sloper 1955) and diffusion will disturb the result in a limited degree only during the first 15 to 30 minutes of incubation, after which it becomes more marked. The method employed to demonstrate alkaline phosphatase, too, is obviously susceptible to the influence of diffusion (Jacoby and Martin 1949). However, it has been found that better results are obtained in regard to alkaline phosphatase after paraffin embedding than in regard to acid phosphatase (Stafford and Atkinson 1948).

Despite the obvious technical shortcomings of the methods employed one is probably well justified in considering the obtained results very likely at least. As Sloper (1955) expresses it, »our attempts at enzyme localization are at best crude; positive results were of some value, but negative results must be regarded with the greatest reserve».

In comparison with previous investigations our results show some disparity with regard to the various enzymes.

Leduc and Wislocki (1952) were able to establish activity of acid phosphatase in the S.C.O. of the animal species investigated by them, but this required an incubation time of 24 hours for the cytoplasmic reaction and of 36 hours for the nuclear reaction to be demonstrated. This difference is probably due to differences in the techniques employed.

In their above-mentioned investigation, Leduc and Wislocki were not able to show any activity of alkaline phosphatase in the S.C.O. although they used the same method. In spite of five hours' incubation no reaction was observed anywhere else but around the blood vessels in the region of the S.C.O. The vascular reaction is thus the same in both sets of results. It seems obvious that this divergence of the results of investigation has to be interpreted as being due to inter-species variations in this respect; differences caused by effects of incubation time, such as have been reported e.g. by Moe (1952), can hardly be concerned since a similar reaction was obtained in both investigations in the walls of the blood vessels. The negative result obtained by Bargmann and Schiebler (1952) in the dog is probably due to the differences in the techniques employed.

The succinic dehydrogenase activity was either found to be entirely absent by Leduc and Wislocki (1952), or there was a very slight reaction in the S.C.O. of the rodents. In the present work the reaction proved to be constantly observable in all animal species investigated. This difference may be attributable to the fact that the neotetrazolium technique. which was used in the present work, has proved to be more sensitive than that with ditetrazolium, which was employed by Leduc and Wislocki (Shelton and Schneider 1952). Our result is in agreement with that obtained with the rabbit by Shimizu, Morikawa and Ishi (1957). The change of colour of the tissue of the posterior commissure observed when the preparations were kept in storage would seem to be a phenomenon similar to that observed by Padykula (1952) and attributed by her to the dissolving of diformazan in lipids.

Activity of non-specific esterases was observed by Leduc and Wislocki (1952) in small rodents by the same method which yielded a positive result with domestic animals in the present work.

No definite physiological rôles can be assigned to the enzymes

in the S.C.O. any more than this can be done with certainty in regard to any enzymes contained in tissues in general. It is merely possible, on the basis of the biochemical properties of the enzymes, to present some conjectures relating to their tasks in the tissue.

Acid phosphatase is a dephosphorylating enzyme needed in the cells in connection with processes requiring great quantities of energy. By its mediation the release of the energy of the phosphate bonds takes place. This also applies to alkaline phosphatase. The contribution of the latter to specific cell functions is still problematic as is that of the other enzymes as well, but Rosenberg and Wilbrandt (1952) have suggested that it would be important with a view to the mechanism of the transmembranic migration. In general one should be able to say that the phosphatases »may be concerned either with maintenance of the cells themselves or with the production of the specific secretory products of the cells» (Leduc and Wislocki 1952). For instance, the rich acid phosphatase content of the hypothalamic ganglia in the central nervous system has been thought to constitute an indication of the protein synthesis occurring in these cells (Eränkö 1951 b, LaVelle, Liu and LaVelle 1954). In these cells obviously a synthesis of the principles of the posterior pituitary takes place.

The biochemical properties of the histochemically demonstrable non-specific esterases seem to indicate that they would play a rôle in some stage of the lipid metabolism (Ammon and Jaarma 1950). These enzymes hydrolyse the esters of fatty acids excepting acetylcholine (Bloor 1943, Mendel and Rudney 1943). Kaplan, Cohn, Levinson and Stern (1939) have found the cerebrospinal fluid to contain such esterases. Indeed, Leduc and Wislocki (1952) put forward the assumption that they would have their origin, partly at least, in the tissue of the S.C.O.

According to present-day conceptions, succinic dehydrogenase is the effective enzyme in the citric acid cycle of Krebs. It is encountered in greatly variable tissues also in histochemically demonstrable quantities (Keilin and Hartree 1949, Padykula 1952). The enzyme is closely bound to the cell, being possibly associated with the mitochondria (Harman 1950). Its significance is obviously connected with the cell's own energy metabolism. According to Padykula (1952), »the distribution of this enzyme suggests that this oxidative enzyme is present in greatest con-

centration within those portions of tissues and organs which show high metabolic activity, such as muscle, or are engaged in absorptive or secretory activities. Conversely, the enzyme could not be demonstrated in regions which are considered to be less active metabolically».

Although the physiological specific significance of the particular enzymes in the S.C.O. cannot be defined in detail on the basis of present knowledge, it is perhaps nevertheless possible to draw some inferences as to the metabolic activity of the organ. If it is feasible to demonstrate also histochemically an activity of so many enzymes in some organ, this should no doubt be interpreted as indicating high metabolic activity taking place in the region of this organ.

Synopsis

In all the animals examined histochemically demonstrable activity of acid and alkaline phosphatase, succinic dehydrogenase and non-specific esterases could be observed. The activity of all enzymes manifested itself in the region of the ependymal cells, and in the ox furthermore in the parietal cells of the hypendymal duct structures. The glia-like cell tissue of the hypendyma showed activity of acid phosphatase only. The walls of the hypendymal blood vessels gave a positive reaction to alkaline phosphatase, non-specific esterases and succinic dehydrogenase. No essential differences could be noted between the different animal species. These findings are taken to support the view that intense metabolic processes take place in the subcommissural organ.

5. ON THE LOCALIZATION OF CARBOHYDRATES, LIPIDS AND PROTEINS IN THE SUBCOMMISSURAL ORGAN

Demonstration of the three principal structural materials of tissues, the carbohydrates, lipids and proteins, and study of their distribution, has been the objective of numerous recent investigations. Also some work related to the S.C.O. has been done with the methods that are being generally used to this purpose. Bargmann and Schiebler (1952) noted in the S.C.O. of dog and cat material stainable by the periodic acid-Schiff procedure; this material occurred in the same region as the chrome-alum-haematoxylin-positive material. From this, the authors inferred that the »secretory material» would have the character of a glycoprotein. Similar observations have been made by Wislocki and Leduc (1952 b) with small rodents. Also Mazzi (1952) with lower vertebrates, Oksche (1954, 1956) with an extensive animal material from *Cyclostoma* to man, Fleischhauer (1957) with the tortoise and Wislocki and Roth (1958) with the human foetus established periodic acid-Schiff-positive material in the cells of the S.C.O. Wislocki and Leduc (1952 b) and Fleischhauer (1957) found also lipids in the organ.

The present work had, among others, the objective of localizing with the aid of some generally used methods the said three tissue components in the tissue of the S.C.O. of ox, horse, pig, and dog.

Methods

Carbohydrates. — The periodic acid-Schiff method developed by McManus (1946) was employed. The material was fixed in 10 % formalin, in Orth's fluid or in Zenker's formol, and embedded

in paraffin. Sections were cut at 5 to 10 micra. Part of the material intended to be used as controls was treated in one of the following ways prior to staining: 1) The sections were incubated in saliva for one hour at 37°C, 2) The sections were incubated for 24 hours at 60°C in a methanol-chloroform mixture (1 : 1), 3) The sections were incubated in the manner recommended by Lillie (1954) in 0.1 M phosphate buffer at pH 6 containing 5 mg per ml hyaluronidase (Bios Laboratories), at 37°C for up to 18 hours. Part of these sections had been prepared from material fixed in 95 % ethyl alcohol. In addition to these controls some sections were stained, in view of obtaining a control, without preceding periodic acid treatment.

Some sections were stained with a view to establishing their potential metachromasia. This material was fixed in Carnoy's fluid (Hale 1946) and embedded in paraffin. Staining was performed in a 0.1 % aqueous solution of toluidine blue in the manner presented by Lillie (1954).

Lipids. — The localization of lipids was studied by means of two methods:

1) Sudan black B staining was performed, staining the frozen sections with the staining agent satured in 70 % ethyl alcohol. The staining time was 7 minutes.

2) Baker's acid haematein method for the demonstration of phospholipids was applied both before and after pyridine extraction (Baker 1946). The material was fixed in Baker's calcium chloride-formalin (Baker 1944), and sectioned with the freezing microtome.

Proteins. — The following methods suitable for the demonstration of certain amino acids or sylphhydryl groups bound to proteins were employed:

1) Sakaguchi's reaction for the demonstration of arginine was used according to Baker's (1947) modification. The material was fixed either in Bouin's fluid or in absolute ethyl alcohol.

2) The ninhydrin test for the demonstration of α-amino acids was used in the manner described by Serra (1946). Fixation was performed either in 10 % formalin or in Bouin's fluid.

3) Millon's reaction for the demonstration of tryptophan and tyrosine was employed in the form developed by Serra (1946). Fixing was performed in absolute ethyl alcohol.

4) In principle according to the original Alcian blue method,

that of Adams and Sloper (1956) was employed to demonstrate cystine. However, 0.25 % potassium permanganate was used as an oxidizing agent and the sections were subsequently treated with 2 % oxalic acid until they were colourless. Staining was performed with 0.5 % Alcian blue at a pH of 0.2 and at room temperature for one hour. Haematoxylin was used for counterstaining. Some sections were stained without preceding oxidation.

5) The dihydroxy-dinaphthyl-disulphide method for the demonstration of sulphhydryl groups bound to protein (Barrnett and Seligman 1952) was used in its original form in all other respects except that the material was fixed in 10 % formalin, as recommended by Gomori (1956).

6) The p-chloromercuriphenyl-azo-β-naphthol method for the demonstration of sulphhydryl groups bound to protein was applied in the form developed by Bennett (1951). The material was fixed in 10 % formalin (Gomori 1956) and the staining time was 18 to 24 hours.

Results

Carbohydrates. — Periodic acid-Schiff (PAS)-positive material could be noted fairly abundantly in the region of the S.C.O. In the ependymal cells the PAS-positive material occurred partly as very finely divided granules, partly as a homogeneous mass. The material was cytoplasmic. Most profusely it was present in the apical part of the cells, condensing to a homogeneous mass at their apical limit. The basal cell processes, too, contained strongly staining material. In the parietal cells of the hypendymal ducts of the ox the material was particularly accumulated in the part of the cells adjacent to the lumen. On the free surface of the organ the PAS-positive material formed a granular mass with condensations here and there, resembling Reissner's fibre. In the ox the lumina of the hypendymal ducts and the capillary spaces extending from there into the cellular interstices were filled with a granular or homogeneous, strongly staining mass (Fig. 29).

The walls of the blood vessels were stained both in the region of the ependyma and of the hypendyma. Similar staining could also be observed in the region of the surrounding brain tissue.

In the sections pretreated with saliva, methanol-chloroform

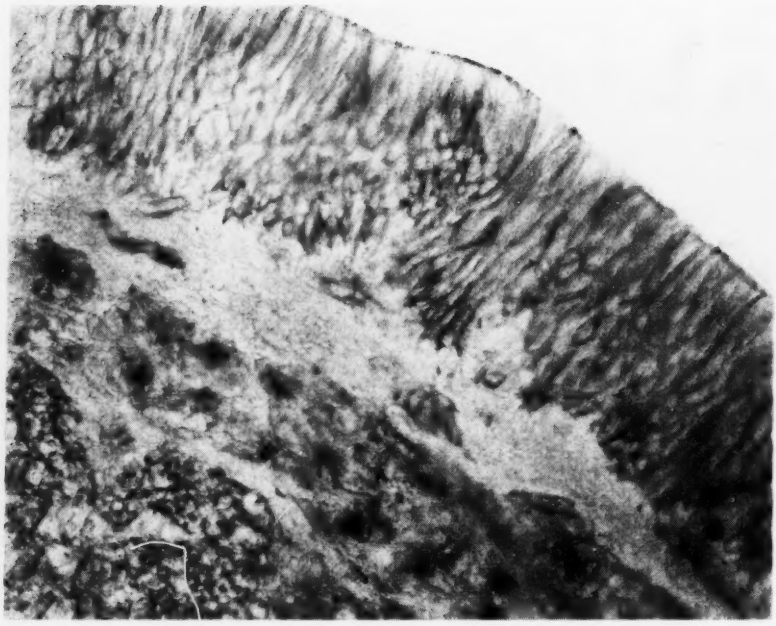

Fig. 29. Ox. — Periodic acid-Schiff-positive material in the ependyma and in the hypendymal ducts. — McManus' method upon saliva incubation. Magnification ca. 400 ×.

mixture or hyaluronidase the result was completely in conformity with that described above, whereas no positively reacting material could be established without periodic acid oxidation.

No material staining metachromatically with toluidine blue could be established in the cells of the ependyma nor of the hypendymal ducts of the ox.

Lipids. — In the region of the S.C.O. proper there was material acquiring a blackish grey stain with Sudan black B (Fig. 30), whereas the posterior commissure was stained strongly black. The grey-stained material occurred diffusely in the ependymal cells and in the ox also in the parietal cells of the hypendymal ducts. The material in these ducts and on the free surface of the organ took on a similar greyish stain. The sudanophilic material in the ependymal cells was located particularly in their apical part and in their basal processes.

Fig. 30. Ox. — Longitudinal section of the S.C.O. Below, the posterior commissure. — Sudan black B. Magnification ca. 400 ×.

Baker's phospholipid method produced strong staining of the posterior commissure. In the region of the S.C.O. stainable material occurred in the same areas as the material acquiring a grey stain by the Sudan method (Fig. 31). Upon pyridine extraction this material could be noted to have disappeared completely (Fig. 32). The cell nuclei could be made clearly visible upon extraction.

Proteins. — Sakaguchi's and Millon's reactions and the ninhydrin test all produced a similar result. They caused diffuse staining of the entire S.C.O. and it was not possible to reveal any particular structures with their aid.

The Alcian blue technique revealed the localization of intensely stainable material in the region of the S.C.O. Without preceding oxidation only the walls of the blood vessels were stained blue, but after oxidation positively stainable material was encountered in the cytoplasm of the ependymal cells, particularly in the apical parts and in the basal processes in all the animals examined (Fig. 33). The surface of the organ was also covered by similarly

Fig. 31. Ox. — Distribution of phospholipids in the S.C.O. Below, the posterior commissure. — Baker's acid haematein method. Magnification ca. 400 ×.

stainable material. In the ox the positive material occurred both in the cytoplasm of the parietal cells and in the lumina of the hypendymal ducts (Fig. 34). Occasionally, the positive material was found to be distinctly granular.

When treated according to Barrnett-Seligman's method, the organ was clearly stained (Fig. 35). In all animals the entire cytoplasm of the ependymal cells was stained red, although the colouring was strongest in the apical parts of the cells and in their basal processes. In places the colour was slightly bluish in these parts. The stained material was a finely divided, granular mass in some places. The nuclei were stained, too. The parietal cells of the hypendymal ducts in the ox were similarly stained and their most strongly stained part was that adjacent to the lumen of the duct. The mass in the free space of the cerebral ventricle together with its fragments obviously formed of Reissner's fibre was stained granularly to a red colour, as was also

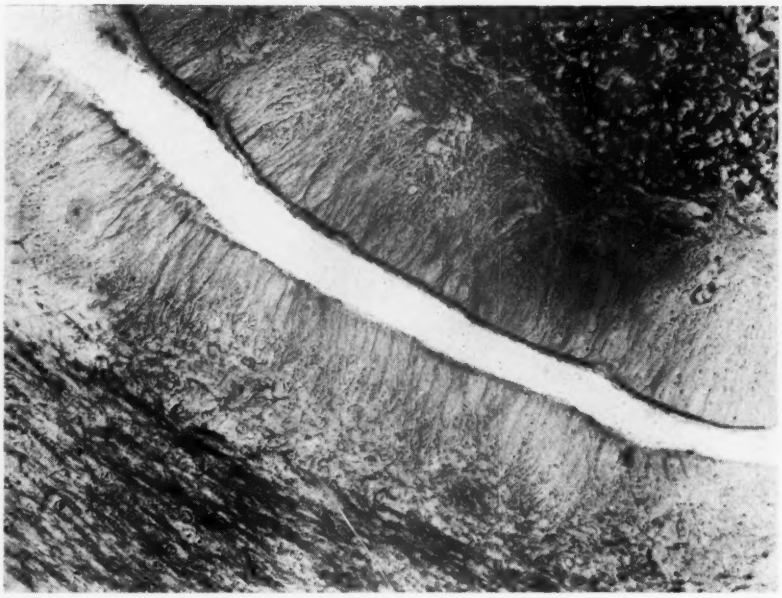

Fig. 32. Ox. — Longitudinal section of the S.C.O. — Baker's acid haematein upon pyridine extraction. Magnification ca. 200 ×.

the material in the lumina of the ox's hypendymal ducts. The walls of the blood vessels acquired a red colour in places.

By Bennett's method a similar result was obtained as by the preceding method (Fig. 36). However, the staining had a somewhat more diffuse appearance and no equally distinct structures could be made visible, yet the reaction was distinct with this method too. The differences in staining intensity in various parts of the cells were completely the same.

Discussion

Most of the methods employed produced a positive result in a region corresponding to the area of distribution of the previously investigated »secretory material». The positively reacting material revealed by several methods had a granular structure.

In the PAS method the positive result is due to the aldehyde

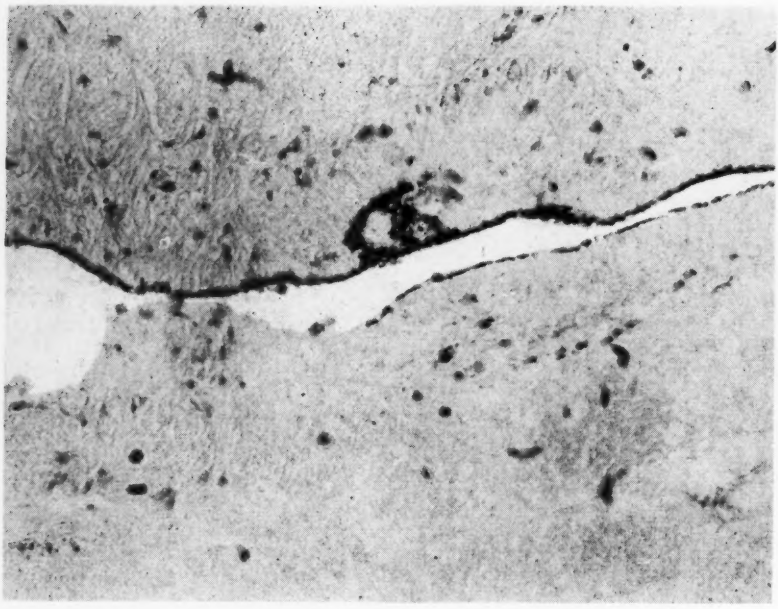

Fig. 33. Dog. — Portion of the ependyma. — Alcian blue without counterstain. Magnification ca. 300 ×.

group produced by the oxidizing action of the periodic acid. Such aldehyde groups are formed in the tissue by the 1.2-glycol groups, the 1-hydroxy-2-amino groups, the 1-hydroxy-2-alkyl-amino groups and the oxidation products of the glycol group (Lipp 1954). These groups occur in numerous different substances. Among the water-soluble substances the monosaccharides, amino acids and some proteins, among substances insoluble in water the polysaccharides, proteins, carbohydrate-protein complexes and some fats and substances soluble in fat solvents show positive reaction (Leblond 1950). In view of the employed fixing methods, however, the reaction produced by water-soluble substances, monosaccharides, amino acids and some proteins need not be taken into account since all fixing fluids contained water. Fats and substances soluble in fat solvents were eliminated by using a methanol-chloroform incubation, which is an adequate procedure for this purpose, according to Gersch (1949). Free polysaccharides, such as the most important carbohydrate insoluble in water in the animal organism,

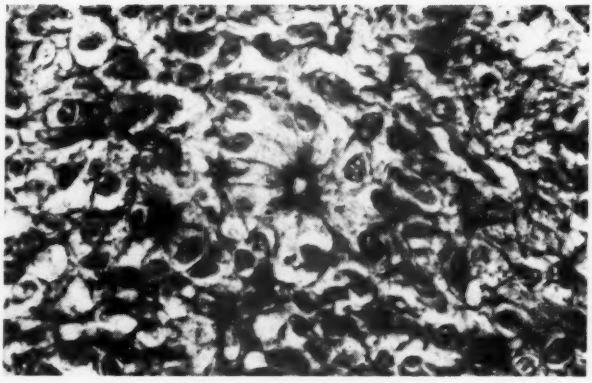

Fig. 34. Ox. — Hypendymal ducts. — Alcian blue + haematoxylin counterstain. Magnification ca. 600 ×.

glycogen, are decomposed to water-soluble products by saliva treatment. In the control tests this treatment did not change the effect obtained, and it can therefore hardly be due to glycogen. Consequently, there remains the alternative that the substance producing positive PAS reaction in the S.C.O. is probably a protein or a protein-carbohydrate complex. As a rule, however, the principal constituent in tissue reacting positively to PAS under the present conditions is considered to be made up of polysaccharides either occurring free or bound to protein since pure proteins rarely produce a reaction (Hotchkiss 1948). An acid mucopolysaccharide which could contain sulphate groups can hardly be concerned as no metachromasia could be established (cf. e.g. Lison 1935). Nor does the substance appear to be hyaluronic acid since hyaluronidase did not alter the stainability.

In comparison with earlier results obtained by the PAS method with various animals the present result shows full agreement.

The result obtained with Sudan black B staining does not exhibit fully the typical dark colouring characteristic of fats. The colour was lighter, of a greyish shade. Wislocki and Leduc (1952 b) arrived at a similar result with small rodents. These authors state the staining to be such that it can hardly be due to neutral fat or myelin; instead, they think that it is caused by phospholipids.

The present observations on the sudanophilic material and

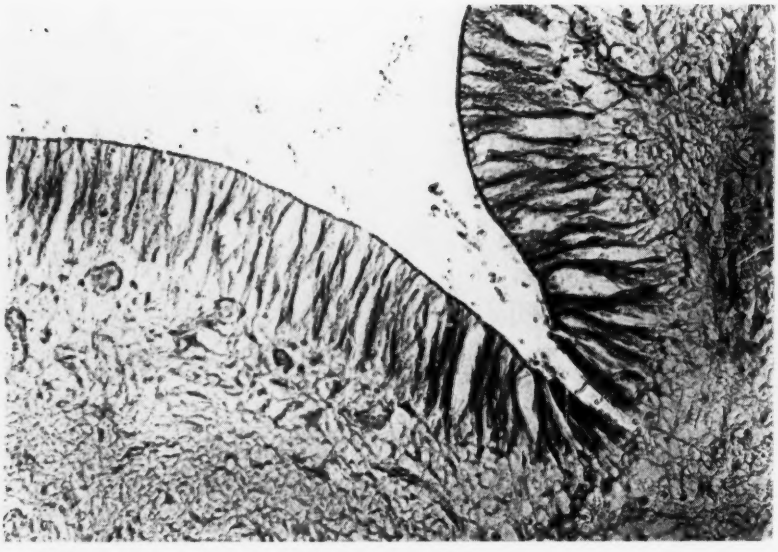

Fig. 35. Horse. — Section of the S.C.O. — Barrnett-Seligman's method for the demonstration of protein-bound sulphhydryl groups. Magnification ca. 200 ×.

the material stainable by Baker's phospholipid stain reveal that both kinds of material are distributed over the same region. This would indicate that the suggestion cited above in regard to the phospholipid character of the sudanophilic material is correct.

No histochemical methods applicable to the demonstration of proteins have yet been developed. Although such substances obviously constitute the principal basis of »living material», it is difficult to demonstrate undisputably the proteins in histological sections and their nature (Lipp 1955). Some staining methods are available for demonstrating certain parts of proteins. Such methods are the stains employed in the present work in our attempts at localization of the proteins. Nothing definite can be said about the results obtained with Sakaguchi's and Millon's reactions and with the ninhydrin test. The entire region of the S.C.O. contains in uniform distribution substances giving a reaction in such degree that the attempts at localization have to fail.

Material stainable by the Alcian blue technique was localized in exactly the same region as the secretory material previously

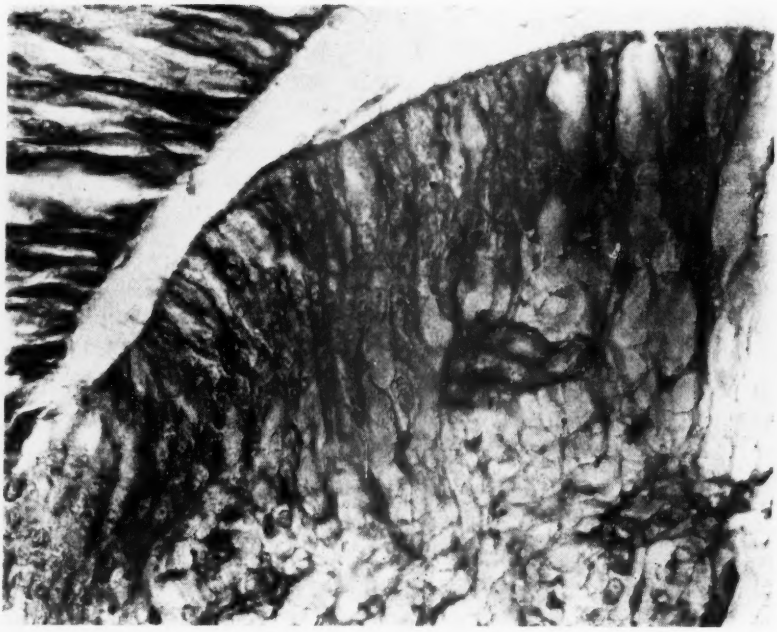

Fig. 36. Horse. — Portion of the ependyma. — Bennett's method for the demonstration of protein-bound sulphhydryl groups. Magnification ca. 600 ×.

referred to in Section 3. According to Adams and Sloper (1956) this staining method is specific for the demonstration of cystine. Without preceding oxidation it was possible to demonstrate positively stainable material only around the blood vessels. This reaction was probably given by acid mucopolysaccharides which were also stained without oxidation as reported by Adams and Sloper (1956). The Alcian blue method is an excellent method for the selective demonstration of the neurosecretory material in the hypothalamo-hypophysial system (Bargmann 1957). The selectivity demonstrable in this manner also appears to exist in the »secretory material» of the S.C.O.

The tests discussed in the preceding are based on the reaction of certain amino acids occurring in the proteins. The reaction showing sulphhydryl groups bound to protein can be said to be a similar, indirect method for the demonstration of proteins. In animal tissues all sulphhydryl groups are thought to be bound to amino

acids, particularly to cystein (Lipp 1956). It is therefore possible with these stains, too, to attempt a localization of the protein. The result obtained can be taken to indicate that the S.C.O. contains proteins possessing sulphhydryl groups. Their concentration seemed to be somewhat variable in various parts of the cells. The cytoplasm of the ependymal cells and in the ox also of the parietal cells of the hypendymal ducts was stained red with Barrnett-Seligman's method as a rule, which constitutes an indication of a small quantity of sulphhydryl groups in the cytoplasm (Barrnett 1953). The sulphhydryl group content, as far as it can be assessed on the basis of the colouring, was highest in those parts where the »secretory material» occurred. In these places even as much as »moderate activity» was present.

Apart from the higher sulphhydryl concentration in the region of distribution of the »secretory material», slight activity also occurred elsewhere in the cytoplasm. Only speculations are possible upon the significance of this observation inasmuch as the function of proteins containing sulphhydryl groups in the cell can be greatly variegated (Barron 1951). They may be parts of enzymatic systems, they are needed in cell division, and they may be linked with cell permeability. Such groups are also necessary in numerous syntheses such as the hormone syntheses. It is obviously not possible to decide between the numerous alternatives on the basis of the present investigation.

On the strength of the findings according to which the intracellular and extracellular »secretory material» of the organ was localized very accurately in the same region as the PAS-positive material, the sudanophilic material probably containing phospholipids and the material containing cystine and most rich in protein-bound sulphhydryls, one is entitled at least to put forward the assumption that the last-mentioned substances would belong to the same »secretory material» and that they would consequently constitute parts of the complex formed by this material. This is supported to some extent also by the granular structure of the material, which was revealed by most of the employed methods.

Synopsis

In the region of the subcommissural organ PAS-positive material, sudanophilic material, material reacting positively to Baker's phospholipid method and protein containing cystine and sulphhydryl groups could be demonstrated, the localization of each one of these substances appearing to be the same both intracellularly and extracellularly. No noteworthy differences between this localization and the region of distribution of the »secretory material» established in Section 3 could be observed. This justifies the tentative assumption that staining reactions of one and the same substance or of substances belonging to one and the same complex would be concerned in each case.

6. EFFECT OF EXTRACTION AND ENZYMATIC DIGESTION ON THE STAINING PROPERTIES OF THE »SECRETORY MATERIAL» IN THE SUBCOMMISSURAL ORGAN

In the present work the term »secretory material» is applied to denote that material which is stained selectively by the methods employed to describe neurosecretory phenomena and reported in Section 3 as having been used in this work. However, these methods are not specific, and it is therefore necessary to use other procedures in order to determine the character of the stainable material.

Analyzing this kind of material presents histochemical difficulties, above all owing to the lack of suitable histochemical methods of investigation. In the present work the aim was set to investigate the influence of the treatment with certain fat solvents upon the staining of the material and the effects of some enzymes on it.

As far as could be ascertained from the literature, no similar investigations have been carried out before with regard to the S.C.O.

Methods

The staining methods applied to the »secretory material» were the same as have been employed in the work described in Section 3.

As fat solvents, 70 % and absolute ethyl alcohol, acetone, pyridine and methanol-chloroform mixtures (1: 1 and 1: 2) were used. Part of the material was treated before and part after fixing, the latter being performed in Bouin's fluid. The sections of 10 micra were treated with each solvent at room temperature as well as at 37°C for 24 hours. With all other solvents except acetone the sections were also incubated for 24 hours at 60°C.

The effect of enzyme digestion was studied employing pepsin, trypsin and ribonuclease.

The material subjected to pepsin digestion was fixed in 80 % ethyl alcohol or in Bouin's fluid. The paraffin sections of 5 to 10 micra were mounted on slides, deparaffinized and hydrated in the usual manner. Incubation was performed in 0.1 n HCl containing crystalline pepsin (E. Merck AG) 1 mg per ml as recommended by Lillie (1954). The incubation time was 15 minutes to 3 hours at 37°C. The control sections were incubated in 0.1 n HCl without pepsin addition.

The material subjected to trypsin digestion was similarly fixed and treated. The incubation solution was 0.01 M phosphate buffer with 0.1 % sodium fluoride and 0.4 % sodium chloride (Lillie 1954), with a pH of 8.5. The solution contained trypsin (E. Merck AG) 1 mg per ml. The material was incubated at 37°C for 15 minutes to 3 hours. Control sections were incubated in buffer solution without trypsin.

The material subjected to ribonuclease digestion was treated in the same manner as the preceding, with the difference that it was fixed in acetic alcohol formalin (Lillie 1949). The incubation solution contained crystalline ribonuclease (Armour & Co) 0.1 mg per 1 ml phosphate buffer solution of pH 6.0. The material was incubated at 37°C for 3 hours, while the control sections were incubated in buffer solution without ribonuclease. In addition to the methods suitable for the description of neurosecretory phenomena, some sections were also stained before or after incubation by Einarson's method (1932).

Results

Treatment of the sections with fat solvents before or after fixation was not found to affect the staining of the »secretory material» in any way. The result was the same with all species of animals and with all staining methods.

In the enzyme digestion tests equal results were obtained with pepsin and trypsin (Figs. 37, 38, 39 and 40). Both enzymes had the effect of obliterating the stainability of the intracellular as well as the extracellular »secretory material». There was merely

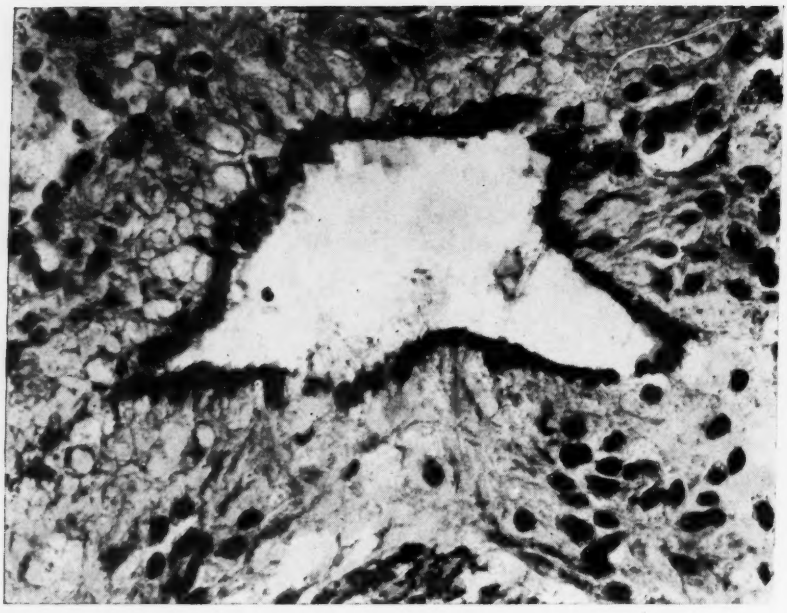

Fig. 37. Dog. — Cross section of a small depression formed by the ependyma. »Secretory material» particularly in the apical parts of the cells. — Aldehyde-fuchsin + haematoxylin counterstain. Magnification ca. 500 ×.

a slight difference in the rate of influence of the enzymes, trypsin showing more rapid effect. With both enzymes, part of the stainable material had disappeared from the sections after 15 minutes' digestion; after 45 minutes' trypsin digestion and 60 minutes' pepsin digestion practically all material had disappeared. Digestion for 30 minutes already showed some signs of damage to the tissue structure. Most resistant to the enzymatic action was the tissue of the posterior commissure. It was not possible to observe any noteworthy differences between the parts of material fixed in different manners.

In control sections incubated in the basic solution without enzyme addition no disappearance of »secretory material» could be noted.

Upon ribonuclease digestion no changes could be noted in the stainable material. However, the stainings done by Einarson'

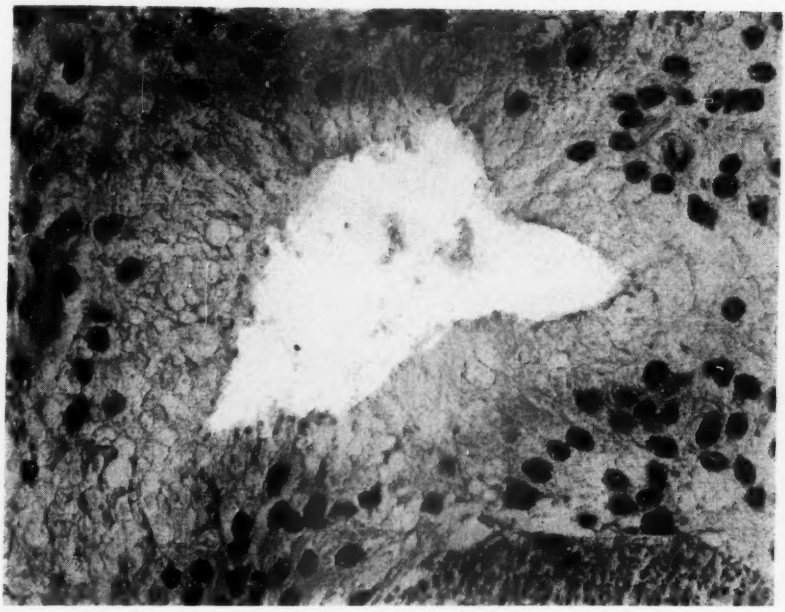

Fig. 38. Dog. — Section from the same points as in Fig. 36. Incubated for 45 minutes with trypsin prior to staining. — Aldehyde-fuchsin + haematoxylin counterstain. Magnification ca. 500 ×.

method showed that in the sections incubated in enzyme solution the stainability of Nissl's substance in the ganglion cells of the brain tissue surrounding the S.C.O. had disappeared.

Discussion

All the solvents used in this instance are solvents generally employed for the dissolving of lipids. According to Folch, Ascoli, Lees, Meath and LeBaron (1951), already the methanol-chloroform mixture will remove under the prevailing conditions the lipids from brain tissue, excepting diphosphoinositide. One should be justified in interpreting this result to the effect that the »secretory material» does not have the character of a lipid or, if it includes a lipid component, that at least this component is not indispensable for the material to be stained. It should be

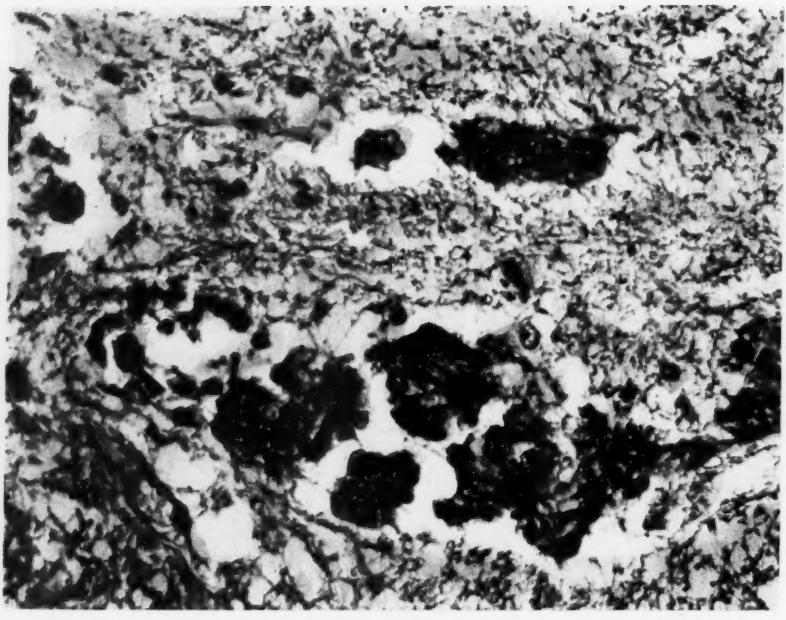

Fig. 39. Ox. — Hypendymal ducts with »secretory material». Below, the posterior commissure. — Aldehyde-fuchsin + haematoxylin counterstain. Magnification ca. 500 ×.

noted that the stainability was not either altered by the pyridine treatment employed earlier to extract the phospholipids (cf. Section 5).

A certain reservation is always indicated in respect to the results of enzymatic digestion tests (Lipp 1955). The results of such tests are susceptible to numerous sources of error, for instance, those referrable to impurity of the enzymes employed or to the disturbing effects of microbes. In the present work it seems unnecessary to take into account any microbial effects, the digestion time being too short for such effects to be able to alter the results in any noteworthy degree. The fixing method may also have influence upon the result. Best results are obtained with alcohol fixation (Krause 1927). However, fixing in alcohol and in Bouin's fluid did not produce any differences in results as to the »secretory material».

Some divergence of opinions exists in respect to the effects of pepsin. According to Mirsky (1943) it decomposes the histones

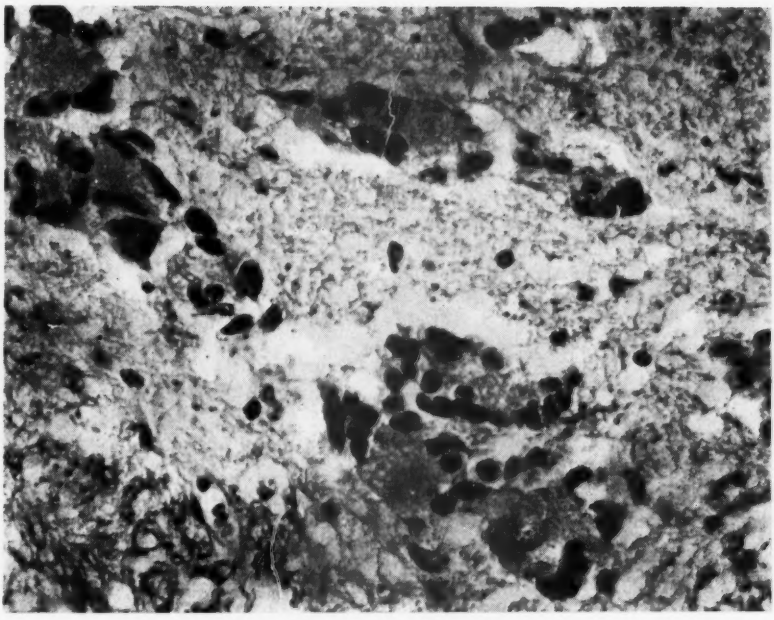

Fig. 40. Ox. — Section from the same point as that in Fig. 39. Incubated for 60 minutes with pepsin prior to staining. — Aldehyde-fuchsin + haematoxylin counterstain. Magnification ca. 500 ×.

of the tissue sections to histopeptones, while Kaufmann, Gay and McDonald (1950) state that the principal effect of pepsin is the decomposition of non-histone type, tryptophan-containing proteins. However, it is unanimously accepted that pepsin belongs to the endopeptidases, affecting the peptide linkages and decomposing protein (e.g. Hawk, Oser and Summerson 1954). Trypsin, too, decomposes proteins and polypeptides in the manner of the endopeptidases. The main effect has relation to the basic proteins but also non-basic proteins are decomposed. Histones are particularly susceptible to its influence (Mazia, Hayashi and Yodowitch 1947).

The digestion tests performed with pepsin and trypsin speak in favour of the idea that the »secretory material» has protein character. The loss of stainability of the material can hardly be explained in any other way than as being caused by structural changes of the material under the influence of the enzymes. The

»secretory material» would thus consist entirely of protein, or it would be a protein-containing complex, with the integrity of its protein part an indispensable prerequisite for its selective staining.

According to Lillie (1954), »the definitive test for the histochemical identification of ribonucleic acid is its digestion by the enzyme ribonuclease». Since in the performed tests ribonuclease had no effects on the stainability of the »secretory material», this material can hardly be ribonucleic acid. It has been established previously (Bargmann 1949, Howe and Pearse 1956) that probably Nissl's substance, which contains ribonucleic acid (Landström, Caspersson and Wohlfahrt 1941), exhibits similar stainability with chrome-alum-haematoxylin as the »neurosecretory material» in the hypothalamo-hypophysial system. On the strenght of this test one should be justified in excluding the possibility that the staining material in the S.C.O. would be ribonucleic acid. The activity of the enzyme on Nissl's substance in the ganglia of the surrounding brain tissue shows that the negative result in the S.C.O. cannot have been caused by inhibition of the enzymatic action for one reason or another under the present experimental conditions.

No corresponding investigations have been made before on the S.C.O. It is therefore not possible to make any comparison with other animal species or other methods of investigation. In the hypothalamo-hypophysial system on the other hand the character of the material stainable by the staining methods employed has been studied by similar procedures of investigation (Sloper 1955, Howe and Pearse 1956). The stainable material has been found to be susceptible to pepsin and trypsin digestion and no change of its stainability could be effected with ribonuclease. Sloper has moreover observed that the material is similar in regard to the effect of fat solvents. This goes to show that both kinds of material exhibiting similar staining by selective methods and found in the central nervous system in two mutually separated systems have similar properties. Considering the comparative crudeness of the methods that have been employed, it is yet not possible to draw the conclusion from these results that both kinds of material would have a similar structure.

Synopsis

The staining properties of the material in the subcommissural organ stainable selectively by the methods employed to describe neurosecretory phenomena have been studied with the aid of extraction with some lipid solvents and by enzymatic digestion. The lipid solvents had no effect upon the staining of the material. Pepsin and trypsin digestion obliterated the stainability of the material in a short time. Ribonuclease had no effect on it. From the results the conclusion can be drawn that the selective stainability of the »secretory material» is not due to the lipid or ribonucleic acid character of it or of some part of it but that probably the staining is dependent on the intactness of the protein occurring at least as a part of this substance.

GENERAL DISCUSSION

In the foregoing sections devoted to the various series of investigation the results obtained in the present work have been discussed particularly in view of the employed methods of investigation. In the following a discussion shall be made of the same results with particular reference to the objectives of study stated in the introduction.

Observations have been made regarding such characteristics in the structure of the S.C.O. which are significant on contemplating the function of this organ. On the whole, these observations can be considered to be in support of a glandular character of the S.C.O. In favour of the other possible alternative functions previously suggested in the literature at least no equally convincing proof can be presented.

No sensory cells could be noted in the region of the S.C.O. This is in agreement with previous results of investigation (Kolmer 1921, Pesonen 1940 a). Consequently that opinion appears less likely correct according to which the organ would act as an intracerebral sensory organ. The nerve fibres which have been found in the hypendyma and which have been considered to advocate this view can be plausibly interpreted in other ways as well.

The selectively stainable material in some cells of the S.C.O. and its localization are clearly indicative of a glandular character of the organ. The localization of this material also outside the cells can be explained by the hypothesis that this material is a secretion. It is notable that in the same cells where this material was encountered, also enzymatic activity of several histochemically demonstrable enzymes was present. Such activity can hardly

imply anything else except a lively metabolic process taking place in the cells. As has already been mentioned, this can be applied to strengthen the opinion that a secretory activity would take place in the cells. On the other hand it is possible to find a similar, strong enzymatic activity in organs in which there occurs resorption (e.g. Padykula 1952). The localization of the selectively stained material in the cells and on their outside might also be explained on the basis of a hypothesis supposing resorptive action to occur. Previously at least Krabbe (1925) has presented a tentative opinion of resorptive action of the S.C.O. However, it is a fair implication that if Reissner's fibre would be resorbed in the S.C.O., it should then be synthetized in the central canal of the spinal chord. Yet, in previously performed investigations no similarly stainable material could be established in the ependymal cells of the central canal, which would be the only conceivable place of occurrence of such a synthesis (Wislocki, Leduc and Mitchell 1956). Furthermore, Reissner's fibre has been observed e.g. in the chicken embryo (Wingstrand 1953) to grow in the cranial to caudal direction, which speaks strongly in favour of its synthesis in the S.C.O.

In the S.C.O. particularly the ependymal cells are resemblant of glandular cells. The parietal cells of the hypendymal ducts of the ox were similar, too. This probably implies that in the ox the hypendyma, which has previously been considered merely some kind of supporting tissue to which no special function could be assigned, is actually a place of occurrence of glandular secretion. Previously, in connection with a morphological investigation on the bovine embryo (Turkewitsch 1936 b), the hypendymal tissue has been found to be embryologically closely related with the pineal body, a fact which is highly interesting in this association in view of its functional character.

On the basis of the results of investigation the most likely assumption concerning the function of the S.C.O. is that of glandular secretion occurring in the organ. The secretion obviously takes place in all the animals in the ependymal cells and in the ox moreover in the parietal cells of the hypendymal ducts, in which the selectively stainable »secretion» and the enzymatic activity are concentrated. The transport of the »secretion» directed into the cerebral ventricle and possibly also directly into the

circulation has been discussed in more detail in Section 3. These opinions are based on the observations made with relation to the morphology of the ependymal cells and the distribution of the material.

The results obtained in the study of the character of the »secretion» are merely orientative, owing to the crudeness of the methods employed. It would seem to have the fundamental character of a protein, containing cystine and bound sulphhydryl groups. On the strength of the localization of the PAS-positive material in the same region where the »secretion» was found, one might think that the protein is associated with a polysaccharide component, and the »secretion» would then be mucoprotein or mucopolysaccharide on the basis of the results obtained in Section 4. The material revealed by the phospholipid staining, which is obviously the same as the sudanophilic material encountered in the region, may hardly be involved in the complex. In that case, however, the presence of phospholipids does not seem to be an indispensable prerequisite for the selective staining of the »secretion».

In the »secretion mass» on the free surface of the S.C.O. dense fibre formations have been observed, which were probably fragments of Reissner's fibre and which exhibited the same staining properties as the »secretion». This can be interpreted to the effect that the fibre is made up of the »secretion», an opinion that has been presented before by some authors (e.g. Wislocki and Leduc 1952 b). However, the possibility that the S.C.O. also discharges its secretion directly into the circulation would tend to imply that forming Reissner's fibre is not the sole objective of the »secretion». This is also indicated by the observation that the S.C.O. contains selectively staining material even in those animals in which Reissner's fibre is lacking (Murakami, Ban and Aiura 1957, Wislocki and Roth 1958) and by the results obtained by Gilbert (1956, 1957) in his experimental investigations, according to which the S.C.O. acts as an organ regulating thirst.

SUMMARY

The objective of the present investigation was to furnish a description of the histological structure of the subcommissural organ in ox, horse, pig and dog and of some of its histochemical properties. Particular attention was paid to such findings which might have significance in relation to determining the problematic function of the subcommissural organ.

In the microscopic structure of the subcommissural organ the most important findings in regard to the function of the organ are thought to be the presence of granular cytoplasmic material in the ependymal cells of the organ in all the animals, and the goblet-like distension of some ependymal cells. These facts are indicative of a secretory activity in these cells. The most essential inter-species difference was the varying length and shape of the basal processes of the ependymal cells. These processes seemed to form a dense network around the subependymal capillaries. In the ox a great number of duct-like structures obviously opening onto the free surface of the subcommissural organ could be established in the hypendyma, resembling the ducts of a tubular gland. In the parietal cells of these ducts similar granular material was encountered as in the ependymal cells. Granular material was also observed on the free ventricular surface of the subcommissural organ and in the ox furthermore in the lumina of the hypendymal ducts. Among the material on the free surface of the organ there were obviously fragments of Reissner's fibre, which had no direct connection with the ependymal cells. Profuse vasculature was noted in the subcommissural organ, particularly in its hypendyma. All animals also exhibited nerve fibres in the

hypendyma. No sensory cells could be found in the region of the subcommissural organ.

By means of the staining methods selectively demonstrating the neurosecretory phenomena in the central nervous system, stainable material could be demonstrated in the subcommissural organ in the ependymal cells of all the animals examined, and in the ox also in the parietal cells of the hypendymal ducts. The localization of the material in the cytoplasm of the cells, on the free surface of the ependyma and in the lumen of the ox's hypendymal ducts are thought to support the opinion of a secretion taking place into the cerebral ventricle and possibly also directly into the circulation. In the material on the free surface of the ependyma fragments probably formed of Reissner's fibre and exhibiting similar staining properties were encountered. In the hypendyma of the horse macroglia-like cells were encountered, the cytoplasm of which contained finely divided, selectively staining material.

By means of histochemical methods, activity of several enzymes could be established, i.e., that of acid and alkaline phosphatase, succinic dehydrogenase and non-specific esterases, in the ependymal cells of all the animals examined, and in the ox also in the parietal cells of the hypendymal ducts. This is thought to speak in favour of a lively metabolism in the organ, of which also its rich vascularization is indicative. In the glia-like cells of the hypendyma, too, acid phosphatase activity was observed in all the species of animals. In the walls of the blood vessels in the subcommissural organ alkaline phosphatase, succinic dehydrogenase and non-specific esterases were demonstrated.

On investigation of the »secretion» of the subcommissural organ results were obtained which tend to imply that the »secretion» has the character of a protein. It gives histochemical reactions indicative of sulphhydryl groups and cystine. The »secretion» loses its selective stainability even upon short incubation with pepsin or trypsin. Ribonuclease does not affect its stainability, nor does the extraction with lipid solvent employed in this work. However, in the region of distribution of the »secretion» in the subcommissural organ sudanophilic material was observed, which is probably phospholipid. It is obvious that if the »secretion» also comprises a lipid component, the presence of this component is

no necessary prerequisite of selective staining. In the region of the »secretory material» there was also periodic acid-Schiff-positive material, which was probably formed by a protein-carbohydrate complex, perhaps consisting of mucopolysaccharide or mucoprotein. On account of the coincidence of localization the possibility cannot be excluded that this material, too, would be involved in the selectively stainable »secretion».

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